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A WIDE RANGE VACUUM TUBE VOLTMETER CIRCUIT[1]

BY G. A. WOONTON[2]

Abstract

The conventional type of vacuum tube voltmeter has been found to be unsatisfactory when used to check amplifiers designed to reproduce e.m.f's whose fundamental frequencies range from a few to 100 cycles per second. These difficulties can be overcome by the use of a symmetrical tube circuit which integrates the two halves of the input wave to produce a smoothed direct current component. Certain other advantages which result from the use of the same circuit are reported. The choice of Type 31 tubes makes the use of an inexpensive plate current meter possible.

Introduction

This investigation was undertaken as part of a general program intended to facilitate the application of physical equipment to medical research.

The application of vacuum tubes to the investigation, recording, and reproduction of an ever increasing number of phenomena has led to the design and construction of amplifiers intended to satisfy a wide variety of conditions. In all cases, amplifiers may be checked and calibrated with the greatest facility, through the medium of the vacuum tube voltmeter.

The conventional type of vacuum tube voltmeter has been found to be unsatisfactory when used to measure the output of amplifiers designed to operate at very low frequencies, or to reproduce exceedingly asymmetrical wave forms. In many cases these amplifiers are used to drive some type of oscillograph, the input to which usually ranges from a few volts up to 100 volts. Since reflexing destroys the square law characteristic of a vacuum tube voltmeter, *i.e.*, introduces wave form error, it is desirable that the voltmeter have a sufficiently extended square law characteristic to allow convenient multiplication of the range up to this value by means of some series resistor arrangement.

[1] *Manuscript received October 1, 1934.*
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Description of the Circuit

With the voltmeter circuit shown in Fig. 1, it is possible to check the performance of amplifiers of widely varying design.

Certain advantages are gained by the symmetrical arrangement of circuit elements. Since only the direct current component of detection flows in the meter circuit, the A-C. components being 180° out of phase, filters and by-passes, which must inevitably introduce both wave-form and frequency error at low frequencies, are not required.

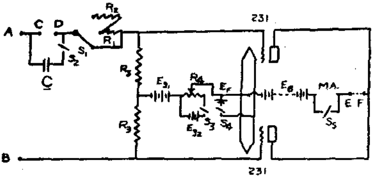

FIG. 1. *The voltmeter circuit.* AB, *input terminals;* CD, *terminals for bucking battery circuit;* EF, *terminals for exterior current meter;* C, *2 microfarads;* S_1, *range switch;* $S_2S_3S_4S_5$, *switches* (S_3 *and* S_4 *ganged);* R_1, *1 megohm;* R_2, *3 megohms;* R_3, *0.25 megohm;* R_4, *2000 ohm variable potential divider;* E_{g1}, *12.5 volts;* E_{g2}, *1.5 volts;* E_F, *1.5 volts;* E_B, *45 volts;* *M.A., 1 milliampere range, Weston model 301 d.-c., milliameter.*

The symmetrical input system fixes the zero axis of the applied input voltage at the cathode potential. The voltmeter automatically integrates the two halves of the input wave, regardless of asymmetry, to give the true effective value.

Since the two grid circuits are effectively in series, the voltmeter will have a square law characteristic over twice the range of applied voltage for a single tube, and at the same sensitivity, since the direct current components of detection are additive in the meter circuit.

It is common practice with all types of effective value tube voltmeters to set the tube bias to give a false zero of plate current. A high value of initial current reduces the percentage error in setting the bias because of the lower percentage error in reading the plate current, but at the same time disproportionately reduces the range over which the tube operates as a square law detector. The circuit illustrated in Fig. 1 gives the same accuracy in setting the bias, for half the plate current per tube, as would be necessary in a single tube circuit, since the plate currents from the two tubes are additive.

The input resistance of the symmetrical circuit is double that for a single tube voltmeter, or viewed in a different way, the resistance in the grid circuit of each tube is just one-half that necessary in a single tube voltmeter to give the same input resistance. A reasonably low grid circuit resistance is important in a voltmeter to be used on either open or short circuited input. It should also be noticed that even on short circuit and for the unmultiplied range the grid circuit resistance can reduce to only one-half of its open circuit value.

Precision

The voltmeter may be calibrated conveniently at 25 or 60 cycles per second, and used to measure potential differences whose fundamental com-

ponents have frequencies as low as 10 cycles per second without special precautions except the introduction of condensers of from 2 to 4 μfd across *CD*. For frequencies between 2 and 15 cycles per second a bucking battery circuit is substituted for the input condenser, if a direct current component flows in the circuit to be measured, and a well damped meter of long period placed across *EF*, the milliameter being shorted out to prevent damage from mechanical shocks.

Since the voltmeter reads the effective value of the applied potential difference, without regard to the sense of the various components, the voltmeter reading must increase when either the bucking battery potential, or the applied direct current component, predominates. The value of the alternating component may be conveniently found by manipulating the bucking battery potential to give a minimum reading on the plate current meter.

It was found by experiment that compensating errors appeared when a false zero current much larger than 0.02 milliamperes was used. With this setting a device for balancing out the zero current is not required. The precision of the instrument was found as:—

6–10 volts, $\pm$ 0.1 volts	2–3 volts, $\pm$ 0.25 volts
3– 6 volts, $\pm$ 0.2 volts	below 2 volts, error extremely large.

On the multiplied scales these errors increase in the multiplier ratio.

These data include errors due both to the operator's inability to read the small panel type self-contained meter closer than 0.01 ma. and to the lack of precision in setting the false zero due to the same cause. Errors entering into the calibration because of lack of precision in the calibrating instruments must be added to these values.

Fig. 2 shows the calibration curve for the instrument, and the precision that may be expected over each of its ranges, including the error introduced in calibrating. These precisions may be somewhat improved if a more accurate current meter is employed in the plate circuit.

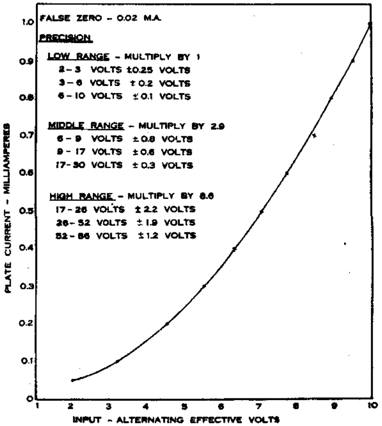

FIG. 2. *Calibration curve—vacuum tube voltmeter.*

Fig. 3 is a plot of plate current against the square of the applied effective volts and shows how accurately the meter follows the desired square law characteristic.

It was found that a uniform drop of ¼ of 1% in calibration could be expected per 1% drop in plate battery potential between 50 and 40 volts. Since the circuit is subjected only to intermittent use, the calibration may be expected to hold accurately for many months. The calibration of the instrument changes very rapidly with changes in filament potential. The filaments of two 231 tubes, connected in parallel as in this circuit, draw approximately 200 ma. at 1.5 volts. It is recommended that two dry cells of standard size be used in parallel to supply the filament current, in order to minimize error from this source. Since the bias is set, by a plate current reading, no error appears in the calibration because of shifts in grid battery potential.

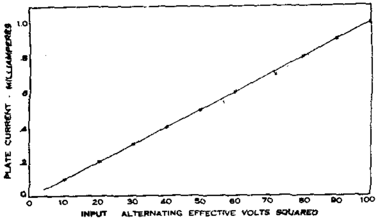

FIG. 3. *Vacuum tube voltmeter calibration curve (exhibiting square law characteristics).*

In order to determine wave form, frequency and turn over error, the meter was calibrated from the commercial mains, which supplies current at 25 cycles per second and whose wave form is a much flattened sinusoid. The meter was then used to measure potential differences across a known resistor due to square waves generated by commutating direct current. The form could be controlled so as to obtain either square unidirectional pulses or a true alternating current.

The results in Table I are typical. They were obtained by holding the effective voltage across the voltmeter constant, while varying the frequency. The voltages employed had a symmetrical, square wave form.

TABLE I

Frequency, c.p.s.	Volts from panel meter	Volts from exterior high inertia meter	Remarks
50	9.8	9.8	
24.5	9.8	9.8	
9.5	9.7	9.7	
1.2	—	9.6	High inertia meter shows ripple of about 2% full scale
50	7.0	7.0	
24.5	7.0	7.0	
9.5	7.0	7.0	
1.2	—	6.9	Ripple approximately 1% of full scale

Turn over and wave form errors were not apparent at any time, even for unidirectional pulsating potential differences.

The ripple which appears in the high inertia meter at these low frequencies has been found to reduce the precision with which the meter may be read to about the same value as that found for the Weston milliameter.

Although this meter has not been checked at high frequencies, its design suggests that it should give excellent results throughout the whole range of audio frequencies and well up into the radio frequency band.

Exceedingly asymmetrical wave forms containing very low frequency components are most commonly met in the investigation of physiological phenomena through their electric effects, such as the e.m.f's accompanying cardiac action, or nervous impulses. In some cases involving the amplification of speech sounds, serious errors in measurement occur through the inability of the ordinary vacuum tube voltmeter to compensate for asymmetry. Although this voltmeter finds its most advantageous application in the cases just mentioned, it has in addition the same range of application as all other types of tube voltmeters, since no new disadvantages are introduced.

The relatively high plate current made possible by the use of the Type 31 tubes allows the use of an inexpensive type of plate current meter. Cheapness, combined with several of the other features enumerated, should make this type of voltmeter especially valuable to the radio amateur.

THE EXCITATION OF BAND SPECTRA—ROTATIONAL STRUCTURE[1]

By G. O. Langstroth[2]

Abstract

An examination of the intensity contours of three second positive nitrogen bands excited by electrons of 14, 15, 16 and 18 electron volts energy, indicates that the contours change in shape as the energy of the exciting electrons is varied. These results and their relation to those of other investigators can be understood if there is a definite probability that an impinging electron will excite the electronic configuration of a molecule and then interact with the rotation before escaping from the molecular field. As might be expected, this probability is appreciable only when the energy of the exciting electron is nearly equal to the excitation energy.

The intensity ratios of the second positive nitrogen bands have been shown to be independent of the energy of the exciting electrons for energies between 25 and 160 electron volts, and the change for energies between 14 and 25 electron volts, if any, is slight (4, 9). It would appear to follow that interchange between electronic translational energy and molecular vibrational energy is improbable under these conditions.

The writer has examined the intensity contours of the bands on his plates in order to see whether there is any evidence of interchange between electronic translational energy and molecular rotational energy, *e.g.*, a dependence of the shape of the band contours on the energy of the exciting electrons. Such a dependence does occur for the bands 0→2 (λ3805), 1→3 (λ3755), and 2→4 (λ3710), for exciting voltages of 14, 15, 16 and 18 volts. The tail intensity is greater, relative to the maximum intensity, the lower the voltage. The change takes place in the same general way for each band.

An approximate analysis of the results indicates that at the lowest exciting voltage the populations of the higher rotational levels are relatively greater than they would be in thermal equilibrium, but that as the voltage is increased they become more nearly those expected for a Boltzmann distribution. Other investigators (7, 8), using similar excitation tubes but higher exciting voltages, have consistently found a Boltzmann distribution in the initial levels.

The observed changes in contour must be connected either with the changes in filament temperature which are necessary to maintain constant tube current as the voltage is varied, or with a direct dependence of the initial level populations on the energy of the exciting electrons. Since there are reasons to believe that the former cannot explain the results, one must look to the latter for the probable explanation.

These results and their relation to the results of others at higher exciting voltages could be understood if a double switch takes place between the energy of the exciting electron and the electronic and rotational energy of

[1] *Manuscript received October 25, 1934.*
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the molecule. This must occur relatively frequently only when the energy of the electron is nearly equal to the excitation energy. Such a process appears to be probable from a consideration of the energy relations.

Some measurements of the intensities of rotation lines with exciting voltages of from 14 to 20 volts and with the proper precautions to eliminate extraneous effects would determine the validity of this explanation, and might lead to some very interesting results.

Experimental

The excitation tube, which consisted essentially of an oxide-coated platinum filament, a guard ring, and a cage with a grid at the filament end, was filled to a pressure of 0.1 mm. of mercury with purified nitrogen, and was operated with tube currents of 0.30 ma. The spectrograph slit was opened to 0.5 mm. to blend the rotational structure completely. Details of the technique are given in former articles (4, 9).

The band contours were determined by measuring the intensity at about 25 points on the microphotometer records for each band by the methods of reference (4, 9). An analyzing apparatus (15) was originally used to do this and later, as a check, straightforward distance measurements were made on new microphotometer records. There was no difficulty in determining corresponding plate distances on the various contours in arbitrary units, and fortunately the change of intensity with plate distance was not great near the tail. No background or interfering bands or lines were present. The contour of λ3805 was determined for exciting voltages of 14, 15, 16 and 18 volts, and two examples are shown in Fig. 1. Measurements were also made at selected points on the contours of λ3805, λ3755, and λ3710 on other plates. Individual relative intensity measurements should be good to within 10%.

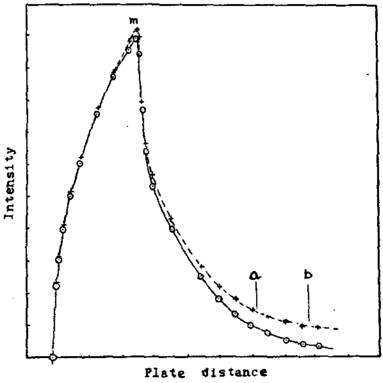

FIG. 1. Comparison of the contour of λ3805 at 18 volts (solid line), and at 14 volts (broken line) accelerating potential. The intensity scale of the former is four times that of the latter. The points represent measured intensities. The plate distances are in arbitrary units which are the same for both contours.

An approximate analysis of the contours can be made if the group of rotational lines responsible for the contour intensity at any point is known. To find it one must determine the broadening (to the violet) of the individual rotation lines

due to the wide slit, their contour, and the frequency which any point on the observed contour would represent if a narrow slit had been used. The broadening was determined from comparator measurements of the width of the mercury lines λ3650 and λ4046 which appeared faintly on the plates. The contours of these lines were flat topped. The "narrow slit" frequencies were determined from measurements on the band edges and the red side of the mercury lines. It was then possible from a consideration of the Fortrat diagram of the rotational structure (2, 5, 6) to assign to each point on the contour the sum of the intensities of certain rotational lines.

Three points on the contour of λ3805 were chosen—the maximum which contained $R(0)$ to $R(12)$ and $P(1)$ to $P(27)$, Point *a* which contained $R(19)$ to $R(25)$ and $P(34)$ to $P(40)$, and Point *b* which contained $R(24)$ to $R(29)$ and $P(38)$ to $P(43)$. $R(n)$ and $P(n)$ include all members of the triplet and Λ-type doublet structure. The intensities of these points will be referred to as I_m, I_a, and I_b.

It was possible to check the allotment of lines in the maximum by calculation of the intensity for different allotments, assuming a Boltzmann distribution in the initial levels. For temperatures in the range under consideration the calculated position of the maximum agreed closely with that found from the plate measurements. This value is very critical as may be judged from the rapid intensity change on either side of it. The allotments for Points *a* and *b* are more easily made owing to the wider separation of the rotation lines in this region.

Contour Analysis

The relative intensities of individual rotation lines can be calculated from their transition probabilities and the distribution of molecules in the initial states. The intensity factors (transition probability multiplied by statistical weight) of the *R* and *P* lines (considered as "unresolved" triplets) were taken as approximately those given by the Hönl-London formulas for a Type *a* molecule (14), *i.e.*,

$$\overline{R}(n) = A(\kappa - 1/\kappa)\ ;\quad \overline{P}(n_1) = A(\kappa+2)\kappa/(\kappa+1), \tag{1}$$

where κ is the rotation quantum number of the *initial* level, and the line numbers n and n_1 are equal to $(\kappa - 1)$ and $(\kappa + 1)$. If the initial level distribution is a Boltzmann distribution as many experimenters have found, it is given by,

$$N(\kappa) = N(o)\ .\ (2\kappa+1)e^{-\frac{B\kappa(\kappa+1)}{kT}} \tag{2}$$

where $N(\kappa)$ and $N(o)$ denote the relative numbers of molecules in the κ and in the lowest level, k the Boltzmann constant, T the distribution temperature, and B the rotation constant which is 1.814 cm.$^{-1}$ for λ3805 (3).

I_m, I_a, and I_b are then approximately,

$$\left.\begin{aligned} I_m &= A\left[\sum_{n=0}^{12} \bar{R}(n) \,.\, F(\kappa) + \sum_{n=1}^{27} \bar{P}(n) \,.\, F(\kappa)\right] \\ I_a &= A\left[\sum_{n=19}^{25} \bar{R}(n) \,.\, F(\kappa) + \sum_{n=34}^{40} \bar{P}(n) \,.\, F(\kappa)\right] \\ I_b &= A\left[\sum_{n=24}^{29} \bar{R}(n) \,.\, F(\kappa) + \sum_{n=38}^{43} \bar{P}(n) \,.\, F(\kappa)\right] \end{aligned}\right\} \quad (3)$$

where $F(\kappa) = N(\kappa)/(2\kappa + 1)$, and the usual relations between n and κ hold.

If a Boltzmann distribution holds, the distribution temperature for a contour can be determined from the measured intensity ratio of, for example, I_m and I_a, and the theoretical curve for the variation of this ratio with temperature (Equations (1), (2) and (3)). The three temperatures which can be obtained from the three measured intensities should agree if the assumption of a Boltzmann distribution is correct. If there are large discrepancies it is possible to obtain an estimate of the direction and magnitude of the variation of the actual distribution from a Boltzmann distribution.

The following values for a temperature of 600°K give an idea of the magnitude of the terms on the right-hand side of Equations (3). The first and second terms in the first, second, and third equations are respectively, 58.5, 116; 15.2, 0.9; 5.9, 0.1. I_m may be taken as characteristic of the populations of low levels since the strongest lines at 600°K are $R(10)$ and $P(11)$, and I_a and I_b are predominantly influenced by the populations of narrow regions from $\kappa = 20$ to 26, and $\kappa = 25$ to 30 respectively.

Results

The values in Table I are averages of the results of two independent observations. Measurements on λ3755 and λ3710 yield similar results.

The intensity-blackening calibration curves were established over the range of intensities given here, by putting two sets of calibration marks on the plate. By no stretch of the imagination can they be so distorted that they give constant intensity ratios for I_a/I_m, I_b/I_m and I_b/I_a.

TABLE I

VARIATION WITH EXCITING VOLTAGE OF INTENSITY RATIOS ON THE CONTOUR OF λ3805

Accelerating potential, volts	I_a/I_m	I_b/I_m	I_b/I_a
14.0	0.135	0.082	0.61
15.0	0.105	0.058	0.55
16.0	0.098	0.046	0.46
18.0	0.085	0.037	0.44

The values in Table II are the averages of the temperatures found from the two independent sets of data, and are followed by the deviation of the individual values from the mean.

TABLE II

DISTRIBUTION TEMPERATURES FOUND FROM THE OBSERVED INTENSITY RATIOS, BY ASSUMING A BOLTZMANN DISTRIBUTION IN THE INITIAL LEVELS

Accelerating potential, volts	$T_1°K\left(\frac{I_a}{I_m}\right)$	$T_2°K\left(\frac{I_b}{I_m}\right)$	$T_3°K\left(\frac{I_b}{I_a}\right)$
14.0	770 ± 50	890 ± 15	1280 ± 0
15.0	650 ± 20	760 ± 10	1030 ± 180
16.0	620	690	750
18.0	570 ± 50	620 ± 20	710 ± 70

Discussion

Table I shows that the intensity of λ3805 near the tail is considerably greater, relative to the maximum intensity, at 14 volts than it is at 18 volts accelerating potential. Moreover there is a progressive change as the voltage is varied between these limits. This can result only from a relative decrease in the populations of the higher rotational levels with increase in exciting voltage. We shall first consider the results of an approximate analysis of the contours, and later the probable causes of the observed behavior.

If the initial rotational level populations are in a Boltzmann distribution, the effective temperatures determined from any three points on a contour should agree (see section on "Contour analysis"). The results in Table II indicate a higher temperature the farther the considered points are from the maximum. At 18 volts the differences are small and may possibly not be significant, but at 14 volts there are very definite differences. The intensity ratios predicted for the Boltzmann distributions which fit one intensity ratio at each voltage, differ from the observed values for the other two by as much as 35%. In addition to this effect, the calculated temperatures increase as the accelerating potential is lowered.

It is not possible to represent the results by assuming two superimposed Boltzmann distributions. All intensity ratios, however, are given to better than 4% by a composite distribution having a temperature θ from $\kappa = 0$ to $\kappa = 20$, and a temperature T_3 (Table II) for the higher levels. Since I_m is predominantly influenced by the populations of low levels, and I_a and I_b by the levels $\kappa = 20$ to 26, and $\kappa = 25$ to 30, it is probably a rough approximation to the actual distribution. The values of θ at 18, 16, 15 and 14 volts are 550, 570, 560 and 600° K. These will be referred to later. The calculated populations of the $\kappa = 9$, 20, 30 and 40 individual levels relative to that of the $\kappa = 0$ level are 3, 7, 150 and 700% greater at 14 volts than they are at 18 volts.

The individual level populations may be expressed in terms of the number of excited molecules, M, in the $v' = 0$ vibration state by treating κ as a variable and integrating Equation (2) from 0 to 19 with a temperature θ, and from 20 to infinity with a temperature T_3. The factor which makes the two parts of the distribution equal at $\kappa = 20$ must be taken into account. The relative value of M at each voltage is known from the total intensity of the vibration band (4, 9). Hence it is possible to compare the populations of any given level at different voltages. For example the $\kappa = 40$ level would have a 50% greater population at 14 volts than it has at 18 volts, although the ratio of the M values is 0.25.

The present results differ in character from those of other experimenters, who used similar excitation tubes but higher exciting voltages. The latter indicate a Boltzmann distribution in the initial levels (7, 8). Moreover it is probable that the distribution temperature is determined by the temperature of the cage walls. Ornstein and Van Wijk, and Van Wijk (10, 12; 13, p. 586), have shown by direct measurement that this is so when the molecules are excited inside a hollow cylinder. Lindh's results indicate an increase of 30 or 40° C. in the distribution temperature for an exciting voltage increase from 150 to 175 volts. If his single intensity measurements were made to within 8%, the greatest accuracy obtainable in the average distribution temperatures was 3% (a possible error of 17° C.). The average values for the relative intensities for each set of conditions varied from the corresponding values for the other sets by less than 6%, with one exception. On the other hand, the two sets of values for the relative intensities for each particular set of conditions showed variations of as much as 8%. One must accept the results, however, as showing some increase in the distribution temperature with increase in exciting voltage. The writer thinks that this is probably due, not to a direct dependence of the rotational distribution on the exciting voltage, but rather to an increase in the cage wall temperature (*cf.* reference 1). The cage temperature is determined by the equilibrium condition between heat gained by electron bombardment, gaseous conduction, and absorption of radiation from the filament, and heat lost by conduction, mainly through the single supporting wire. Owing to the high accelerating potentials used by Lindh, the second and third factors remained practically constant for constant tube current, so that an increase in cage temperature is expected because of the increased energy input. The increase, estimated from the known construction characteristics of the tube, is of the right order of magnitude. It may be that the temperature decrease expected for a decrease in tube current is not apparent because of the rather wide limits of accuracy of the temperature measurements. The experiments of Ornstein and Kruithof on hydrogen (8) furnish additional supporting evidence. They found the distribution temperature independent of the exciting voltage between 20 and 30 volts, but dependent on tube current, *i.e.*, filament temperature. This can be understood from the increased cage temperature (due to increased radiation and gaseous conduction) with increase in filament temperature. When the exciting voltage is decreased under these conditions, the filament temperature must be materially increased and so tends to compensate for the lowered energy input. The fact that these authors found lower distribution temperatures (about 300° K) than those found by Lindh (about 560° K) may result from the lower gas pressure (0.03 rather than 0.1 mm. of mercury) and consequently lower gaseous conduction, which under these conditions depends on the pressure. (In this type of tube the filament is placed as close as possible to the cage grid.)

In contrast to these results the characteristic feature of the present results was a favoring of the populations of high levels at 14 volts, which decreased as the voltage increased, so that the distribution tended to become a Boltz-

mann distribution at about 18 volts. In addition, the higher the level the more it appeared to be favored. The explanation must be sought either in some temperature effect due to the higher filament temperatures necessary for constant tube current at the lower voltages, or in a direct dependence of the relative populations of the initial rotational levels on the energy of the exciting electrons.

An estimate of the increase in the temperature of the cage and the filament when the exciting voltage dropped from 18 to 14 volts was obtained from a duplicate tube. The former was measured with a thermocouple and the latter with an optical pyrometer. Various adjustments of filament, guard ring and cage were made to overcome slight divergences of the duplicate from the original. The maximum filament temperature increase was 200° C. and the corresponding cage temperature increase was 26° C. The latter was probably somewhat higher in the original tube since the pressure was somewhat greater.

The solid angle subtended by the filament at the point from which the observed light came was certainly less than $\frac{1}{100}$ of that subtended by the cage walls. Since the accommodation coefficient for nitrogen is comparable to 1 (13), it is to be expected that the only effect of the rise in filament temperature was to increase the temperature of the cage walls. This view is supported by the work of Ornstein and Kruithof, in which a current variation between 0.2 and 1.5 ma. at constant voltage did not destroy the Boltzmann distribution, but increased the distribution temperature. The filament temperature changes involved were certainly greater than those in the present work. The observed effects might be explained by a remarkable persistence of rotation on collision with a wall for rapidly, but not for slowly, rotating molecules, but this should occur as well in other experiments with similar tubes, and a Boltzmann distribution has consistently been found.

On the other hand, energy intérchange between the impinging electron and the molecular rotation is expected to be probable only when the two energies are comparable. Since the rotational energy of the molecule in the $\kappa = 20$ level of the ground state is only 0.1 electron volt, this is unlikely to be the explanation. The number and character of the slow secondary electrons in the tube probably do not change rapidly enough with the exciting voltage to account for the observed behavior on the basis of their interaction with the molecular rotation.

The observed results as well as their relation to those of other investigators could be understood, however, if there is an appreciable probability of a double switch between the energy of the exciting electron and the molecular electronic and rotational energy. One can think that an electron after exciting the electronic configuration, has a definite probability of interacting with the rotation before escaping from the molecular field. The energy relations are such as to make this possible. The exciting voltage at which the bands first appear for this set-up is easily obtained from extrapolation of the optical excitation function (4, 9). The value so obtained is 13.4 volts. A 14 volt electron then has an energy of 0.6 electron volt after exciting the electronic

configuration. This is of the order of the rotational energy (0.28 electron volt for $\kappa = 35$), and might be expected to interact with it. As the energy of the exciting electrons is increased the excess energy becomes greater and the probability of interaction consequently less, until a point is reached at which it is improbable. Above this point one would expect to find a Boltzmann distribution.

If this explanation is correct the populations of the low levels should be in an almost Boltzmann distribution with a temperature determined by the cage temperature. In the composite distribution which gives the correct intensity ratios, the low level distribution temperatures, θ, varied from 550° K at 18 volts to 600° K at 14 volts. The increase is of the magnitude of the increase in cage temperature found by direct measurement in the duplicate tube. Moreover the distribution temperatures are about the same as those found by Lindh for the negative band λ3914, with higher exciting voltages but otherwise identical conditions. It is known that λ3914 and λ3805 have the same rotational distribution temperature in the same discharge (12). The θ values seem to be reasonable therefore, in view of the compensating effects of increased filament current and decreased energy input.

Although the evidence for this explanation cannot be regarded as conclusive because of the approximate nature of the analysis, there are many points in its favor. The fact that the departure from a Boltzmann distribution is great at 14 volts and slight (if any) at 18 volts is especially encouraging. Determination of the validity of the explanation must rest on further measurements of the intensities of individual rotation lines.

In conclusion it should be pointed out that all remarks made in this article, especially those on page 11, refer *only* to the excitation of molecules by electronic impact. When there are also collisions between molecules and heavy particles moving under the influence of an electric field, the problem becomes extremely complicated, and the distribution temperature in the excited states may be expected to be related in no simple way to the actual gas temperature [*Cf.* reference 11].

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STANDARD NOMOGRAPHIC FORMS FOR EQUATIONS IN THREE VARIABLES[1]

BY F. M. WOOD[2]

Abstract

Equations of the third and fourth nomographic order in three variables have been dealt with and classified. *Equations of the third order may be reduced to one of two standard forms,* $\alpha + \beta + \gamma = 0$ *and* $\alpha + \beta\gamma = 0$, which give alignment charts composed of three straight lines. Equations of the fourth order may also be reduced to one of two standard forms, resulting in charts composed of (*a*) two straight lines and a curve, or (*b*) two scales on a conic, and the third on another curve. Transformations of these four standard forms are given which permit of rapid and easy adjustment of the position and length of the scales for any given example, resulting in a chart of practical utility. Although the underlying theory has been studied by other writers, notably Soreau and Clark, it has possibly never appeared before in such a neat form. On this account, and also because of the standard transformations, it is felt that this article is of particular value.

Standard forms have also been developed for third order equations leading to charts composed of two scales on a conic and a third straight scale, and in conclusion a third type of chart, in which all three scales appear on a single cubic curve, has been standardized. The practical value of the last type is questionable, but the conic charts are of use since we may arbitrarily choose the unit circle, or the rectangular hyperbola, for our conic scales. Final adjustment forms which permit suitable location of the scales in particular examples have been obtained in every case.

Introduction

In this article an attempt is made to standardize for practical use the work which has been done on the representation by alignment charts of equations in three variables. Of particular note in this respect is the work of R. Soreau (5–8) and of J. Clark (1–4). Although the fundamental ideas given here are not original, it is hoped that the method of presentation and the arrangement will carry an appeal, especially to those having occasion from time to time to construct such charts.

The equation

$$f_3(a_0f_1f_2 + a_1f_1 + a_2f_2 + a_3) + \phi_3(b_0f_1f_2 + b_1f_1 + b_2f_2 + b_3) + (c_0f_1f_2 + c_1f_1 + c_2f_2 + c_3) = 0$$

is said to be the general equation of the fourth order. Here f_1, f_2 and f_3 are functions of three independent variables, and ϕ_3 is a function of the same variable as f_3, such that it cannot be expressed as a linear form of f_3, *i.e.*, we cannot express ϕ_3 in the form $d_1f_3+d_2$, where d_1 and d_2 are constants. Also a_0, etc., b_0, etc., and c_0, etc., are constants.

The general third order equation may be written

$$f_3(a_0f_1f_2 + a_1f_1 + a_2f_2 + a_3) + (b_0f_1f_2 + b_1f_1 + b_2f_2 + b_3) = 0.$$

This is a particular case of the third order equation when $\phi_3 = 0$, or can be expressed as a linear form of f_3.

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It has been proved that any third order equation may be represented by an alignment chart composed of three straight line scales, one for each of the variables. In most cases a simple algebraic transformation leads to such a chart, whereas in certain special cases a transcendental transformation employing inverse tangents is required. It has also been proved that any third order equation may be represented by a chart in which one of the variables (any one of the three) has a straight line scale, and the other two have scales located on a conic. The value of this type of chart is apparent when it is stated that a circle may be chosen for this conic, and that the two scales may be shifted on the conic independently of each other to suitable positions. Thirdly, it has been proved that any third order equation may be represented by a chart in which all three scales are located on a single curve, which is a cubic, and that in this case also the three scales may be shifted independently of one another along the cubic.

For fourth order equations it has been proved that two types of chart, mutually exclusive, are possible. In one type two of the scales lie on straight lines (f_1 and f_2) and the third scale ($f_3\phi_3$) lies on a curve. In the other type the two scales lie on a conic and the third scale as before lies on another curve. In the latter type the conic scales may be shifted along the conic, but not independently of each other. Although this does not give the freedom of adjustment present in the third order charts, it nevertheless permits suitable placing of the third scale with respect to the conic.

Soreau has studied the theory for the straight line representation of third order equations, and Clark has studied the fourth order equation, and the conic and cubic representation of the third order equation. Clark developed a neat geometric (synthetic) method of constructing the charts, using the anharmonic properties of rays intersecting on a conic.

In the following analysis, the general equations are subdivided into various types, and a standard form is found for each type, and standard transformations of these forms are developed to give suitable charts for any particular example,—charts in which the three scales, covering the particular range of values of the three variables, will be advantageously placed with respect to one another. The analytical method, with rectangular co-ordinates, is used in preference to the geometric construction of Clark, as it is felt that the latter method is not amenable to standardization, not so simple to study and discuss, and, under certain conditions which may arise in any example, is not so accurate.

Alignment Charts of Three Straight Lines for Third Order Equations

The general third order equation is

$$f_3(a_0f_1f_2 + a_1f_1 + a_2f_2 + a_3) + (b_0f_1f_2 + b_1f_1 + b_2f_2 + b_3) = 0\,. \tag{1}$$

It will be shown that this may be reduced to one or other of the two canonical forms $\alpha+\beta+\gamma=0$ — (A), $\alpha+\beta\gamma=0$ — (B); and that both of these forms result in charts composed of three straight lines. These canonical forms are then adjusted to give suitable position for the scales in particular examples.

It is assumed that the constant $a_0 \neq 0$, so that the term containing $f_1 f_2 f_3$ is present. If such is not the case in any given equation, a division by f_1, f_2, or $f_1 f_2$ will put it in the desired form. It will be found that division throughout by a_0, making the coefficient of $f_1 f_2 f_3$ unity, simplifies the work considerably.

Writing $X_1 = f_1 + \frac{a_2}{a_0}$; $X_2 = f_2 + \frac{a_1}{a_0}$; $X_3 = f_3 + \frac{b_0}{a_0}$ we reduce to

$$X_1 X_2 X_3 + B_1 X_1 + B_2 X_2 + B_3 X_3 + 2P = 0 \ , \tag{2}$$

where $B_1 = \frac{1}{a_0^2}(a_0 b_1 - a_1 b_0)$; $B_2 = \frac{1}{a_0^2}(a_0 b_2 - a_2 b_0)$; $B_3 = \frac{1}{a_0^2}(a_0 a_3 - a_1 a_2)$;

$$2P = \frac{1}{a_0^2}\left[(a_0 b_3 - a_3 b_0) - a_0 a_2 B_1 - a_0 a_1 B_2\right].$$

Now substitute $B_1 X_1 = a\phi_1 + b$; $B_2 X_2 = a\phi_2 + b$; $B_3 X_3 = a\phi_3 + b$.
The new form

$$A_0 \phi_1 \phi_2 \phi_3 + A_1(\phi_1\phi_2 + \phi_2\phi_3 + \phi_3\phi_1) + A_2(\phi_1 + \phi_2 + \phi_3) + A_3 = 0 \ , \tag{3}$$

results, where $A_0 = a^3$; $A_1 = a^2 b$; $A_2 = ab^2 + aB_1B_2B_3$; $A_3 = b^3 + B_1B_2B_3(3b + 2P)$.
(*a*) If in this symmetrical form we apply the conditions $A_0 = A_2$; $A_1 = A_3$ we determine particular values of the constants a and b to satisfy, *e.g.*, $a = \sqrt{P^2 + B_1B_2B_3} = R$; $b = -P$.

Equation (3) then reduces to

$$(\phi_1\phi_2\phi_3 + \Sigma\phi_1)R - (\Sigma\phi_1\phi_2 + 1)P = 0 \quad \text{or} \quad \frac{\phi_1\phi_2\phi_3 + \Sigma\phi_1}{\Sigma\phi_1\phi_2 + 1} = \frac{P}{R} \ .$$

Hence $\frac{\phi_1\phi_2\phi_3 + \Sigma\phi_1\phi_2 + \Sigma\phi_1 + 1}{\phi_1\phi_2\phi_3 - \Sigma\phi_1\phi_2 + \Sigma\phi_1 - 1} = \frac{P+R}{P-R}$; or $\frac{\phi_1 + 1}{\phi_1 - 1} \cdot \frac{\phi_2 + 1}{\phi_2 - 1} \cdot \frac{\phi_3 + 1}{\phi_3 - 1} = \frac{P+R}{P-R}$.

Re-substituting for ϕ_1, ϕ_2, ϕ_3 this becomes

$$\frac{B_1X_1 + P + R}{B_1X_1 + P - R} \cdot \frac{B_2X_2 + P + R}{B_2X_2 + P - R} \cdot \frac{B_3X_3 + P + R}{B_3X_3 + P - R} = \frac{P+R}{P-R} \ , \tag{4}$$

which can be put in the canonical form $\alpha + \beta\gamma = 0$ — (B) if we write

$$\alpha = \frac{P+R}{P-R} \cdot \frac{B_1X_1 + P - R}{B_1X_1 + P + R} \ ; \ \beta = \frac{B_2X_2 + P + R}{B_2X_2 + P - R} \ ; \ \gamma = -\frac{B_3X_3 + P + R}{B_3X_3 + P - R} \ .$$

The symmetry of Equation (4) permits a choice of the functions α, β, and γ. However, it may be used only when $P^2 + B_1B_2B_3 > 0$.

(*b*) When $P^2 + B_1B_2B_3 < 0$, the same analysis as for Case (*a*) may be used as far as Equation (3). At this point, the conditions applied are $A_0 = -A_2$; $A_1 = -A_3$; and the resulting values of a and b are $a = \sqrt{-P^2 - B_1B_2B_3} = R_1$; $b = -P$.

Hence we may obtain $(\phi_1\phi_2\phi_3 - \Sigma\phi_1)R_1 - (\Sigma\phi_1\phi_2 - 1)P = 0$, which results in

$$\frac{\phi_1 + \phi_2 + \phi_3 - \phi_1\phi_2\phi_3}{1 - \phi_1\phi_2 - \phi_2\phi_3 - \phi_3\phi_1} = \frac{P}{R_1} \ .$$

If this is compared with the expansion of tan $(A+B+C)$ in terms of tan A, tan B, and tan C, it is evident that the equation may be written

$$\tan^{-1}\phi_1 + \tan^{-1}\phi_2 + \tan^{-1}\phi_3 = \tan^{-1}\frac{P}{R_1} \ ,$$

i.e.,

$$\tan^{-1}\frac{B_1X_1 + P}{R_1} + \tan^{-1}\frac{B_2X_2 + P}{R_1} + \tan^{-1}\frac{B_3X_3 + P}{R_1} = \tan^{-1}\frac{P}{R_1} \ .$$

Here $\tan^{-1}\dfrac{P}{R_1}$ may be combined with one of the other three to give

$$\tan^{-1}\frac{B_1X_1+P}{R_1}+\tan^{-1}\frac{B_2X_2+P}{R_1}+\tan^{-1}\frac{R_1X_3}{PX_3-B_1B_2}=0\ , \tag{5}$$

which is evidently of the canonical form $\alpha+\beta+\gamma=0$ — (A). This may be used only when $P^2+B_1B_2B_3<0$. The scales for this chart are periodic, being graduated according to the angular measures of $\tan^{-1}\dfrac{B_1X_1+P}{R_1}$, etc., which have a period of 180°. However, this need not lead to confusion in plotting, since the two outer scales may be chosen and adjusted for position first, using preferably the period from 0° to 180°. The third scale (*i.e.*, some period of it) will of necessity fall in between these two outer scales.

(*c*) When $P^2+B_1B_2B_3=0$, and all these coefficients B_1, B_2, B_3, P, differ from zero.

Substitute $\phi_1=\dfrac{B_1X_1}{P}$; $\phi_2=\dfrac{B_2X_2}{P}$; $\phi_3=\dfrac{B_3X_3}{P}$ and Equation (2) reduces to

$$\phi_1\phi_2\phi_3-(\phi_1+\phi_2+\phi_3)-2=0.$$

Substituting further $\phi_1=F_1-1$, and similarly for ϕ_2, ϕ_3, we obtain

$$F_1F_2F_3-(F_1F_2+F_2F_3+F_3F_1)=0.$$

This, on division throughout by $F_1F_2F_3$ and re-substitution, gives

$$\frac{P}{B_1X_1+P}+\frac{P}{B_2X_2+P}+\frac{P}{B_3X_3+P}=1\ , \tag{6}$$

which is of the canonical form $\alpha+\beta+\gamma=0$, if we combine one of the three functions with unity on the right-hand side.

The above three cases are applicable when all of B_1, B_2, B_3, P, differ from zero. Following are the special cases when one or more of these are zero.

(*d*) $X_1X_2X_3+B_2X_2+B_3X_3+2P=0$ (one of the coefficients B_1, B_2, B_3, zero).

Dividing through by X_2X_3, and substituting

$$\frac{B_2}{X_3}=\phi_3-\frac{B_2B_3}{2P}\ ;\ \frac{B_3}{X_2}=\phi_2-\frac{B_2B_3}{2P}\ ;$$

this reduces to

$$\left(X_1-\frac{B_2B_3}{2P}\right)+\frac{2P}{B_2B_3}\cdot\phi_2\phi_3=0 \quad\text{or}\quad \frac{1}{2P}\left(X_1-\frac{B_2B_3}{2P}\right)+\left(\frac{1}{X_2}+\frac{B_2}{2P}\right)\left(\frac{1}{X_3}+\frac{B_3}{2P}\right)=0$$

which may be still further reduced to

$$(2PX_1-B_2B_3)+\left(\frac{2P}{X_2}+B_3\right)\left(\frac{2P}{X_3}+B_2\right)=0 \tag{7}$$

of the canonical form $\alpha+\beta\gamma=0$.

(*e*) $X_1X_2X_3+B_2X_2+B_3X_3=0$ ($P=0$ and one of B_1, B_2, B_3 zero).

Dividing through by X_2X_3 reduces this immediately to

$$X_1+\frac{B_3}{X_2}+\frac{B_2}{X_3}=0 \tag{8}$$

of the canonical form $\alpha+\beta+\gamma=0$.

(*f*) $X_1X_2X_3+B_3X_3+2P=0$ (Two of B_1, B_2, B_3, zero).

Dividing through by X_3, this gives

$$\left(\frac{2P}{X_3}+B_3\right)+X_1X_2=0 \tag{9}$$

of the canonical form $\alpha+\beta\gamma=0$.

(g) $X_1X_2X_3+2P=0$ (All three of B_1, B_2, B_3, zero).

Dividing through by X_3, this gives

$$\frac{2P}{X_3}+X_1X_2=0 \tag{10}$$

of the canonical form $\alpha+\beta\gamma=0$.

The above analysis includes all cases of the third order equation. Any particular example for which an alignment chart is required may be reduced to one of these seven cases, and expressions for α, β, and γ determined in terms of the original three functions f_1, f_2, and f_3. Adjustments of the α, β, and γ scales on the charts to suit particular ranges of value of the three variables will now be studied.

Type I. $\alpha+\beta+\gamma=0$, with three parallel straight scales.

The fundamental determinant form is

$$\begin{vmatrix} 0 & \alpha & 1 \\ 1 & -\frac{1}{2}\beta & 1 \\ 2 & \gamma & 1 \end{vmatrix} = 0 .$$

Here the α scale lies on the straight line $x=0$, graduated $y=\alpha$;

The β scale lies on the straight line $x=1$, graduated $y=-\frac{1}{2}\beta$;

The γ scale lies on the straight line $x=2$, graduated $y=\gamma$.

These scales should be shifted so that they lie opposite one another. To do this we use the transformation

$$\begin{vmatrix} 0 & \alpha & 1 \\ \dfrac{2}{m+1} & \dfrac{-m\beta+n}{m+1} & 1 \\ 2 & m\gamma+n & 1 \end{vmatrix} = 0$$

where we have magnified the γ scale m times with respect to the α scale, and then shifted it up a distance n. If the desired chart is to be rectangular in shape, and the extreme values of the variables α and γ are denoted by the subscripts 1 and 2, we must have $\alpha_1=m\gamma_1+n$; $\alpha_2=m\gamma_2+n$. Solving for m and n, this will give the determinant form which places the α and γ scales opposite each other. The β scale must necessarily be placed suitably between these two.

Should it be desired to magnify the β scale instead of the γ scale, the transformation to use is

$$\begin{vmatrix} 0 & \alpha & 1 \\ 1 & -\dfrac{m\beta}{2}+n & 1 \\ \dfrac{2}{2-m} & \dfrac{m\gamma+2n}{2-m} & 1 \end{vmatrix} = 0 .$$

Owing to symmetry, any two of the three variables may be chosen for the outside scales.

Type II. $\alpha+\beta+\gamma=0$, with three concurrent straight scales.

The fundamental determinant form here is

$$\begin{vmatrix} -\frac{1}{\alpha} & -\frac{1}{\alpha} & 1 \\ 0 & \frac{1}{\beta} & 1 \\ \frac{1}{\gamma} & 0 & 1 \end{vmatrix} = 0 ,$$

and the transformation

$$\begin{vmatrix} \frac{1+k}{m+n-\alpha} & \frac{1}{m+n-\alpha} & 1 \\ \frac{k}{\beta+m} & \frac{1}{\beta+m} & 1 \\ \frac{1}{\gamma+n} & 0 & 1 \end{vmatrix} = 0 .$$

In studying this transformation it is seen that the γ scale lies along the x axis, and the other two scales meet it at the origin. Trial alone will indicate in any example the best values to assign to k, m, and n. The use of k frees us from the necessity of using oblique axes; m and n ensure that the scales do not extend to infinity, and also permit adjustment so that the most important parts of the scales, where greatest accuracy is required, shall lie furthest from the origin.

Type III. $\alpha+\beta\gamma=0$, with two parallel and one inclined straight scale.

The six forms listed below cover this type. If, however, the equation is expressed $-\beta\,.\,\gamma\,.\,\frac{1}{\alpha}=1$, or its reciprocal $-\frac{1}{\beta}.\frac{1}{\gamma}\,.\,\alpha=1$, it is seen that a choice is possible in assigning α, β, and γ to the three variables, and when this is done only the first of the six forms is necessary.

$$(a)\ \begin{vmatrix} 0 & \beta & 1 \\ 1 & \alpha & 1 \\ \frac{1}{1+\gamma} & 0 & 1 \end{vmatrix} \rightarrow \begin{vmatrix} 0 & m\beta+n & 1 \\ 1 & \alpha & 1 \\ \frac{m}{m+\gamma} & \frac{n\gamma}{m+\gamma} & 1 \end{vmatrix} \qquad (b)\ \begin{vmatrix} 0 & \beta & 1 \\ 1 & -\alpha & 1 \\ \frac{1}{1-\gamma} & 0 & 1 \end{vmatrix} \rightarrow \begin{vmatrix} 0 & m\beta+n & 1 \\ 1 & -\alpha & 1 \\ \frac{m}{m-\gamma} & \frac{-n\gamma}{m-\gamma} & 1 \end{vmatrix}$$

$$(c)\ \begin{vmatrix} 0 & \frac{1}{\alpha} & 1 \\ 1 & \frac{1}{\beta} & 1 \\ \frac{1}{1+\gamma} & 0 & 1 \end{vmatrix} \rightarrow \begin{vmatrix} 0 & \frac{m}{\alpha}+n & 1 \\ 1 & \frac{1}{\beta} & 1 \\ \frac{m}{m+\gamma} & \frac{n\gamma}{m+\gamma} & 1 \end{vmatrix} \qquad (d)\ \begin{vmatrix} 0 & \frac{1}{\alpha} & 1 \\ 1 & -\frac{1}{\beta} & 1 \\ \frac{1}{1-\gamma} & 0 & 1 \end{vmatrix} \rightarrow \begin{vmatrix} 0 & \frac{m}{\alpha}+n & 1 \\ 1 & -\frac{1}{\beta} & 1 \\ \frac{m}{m-\gamma} & \frac{-n\gamma}{m-\gamma} & 1 \end{vmatrix}$$

$$(e)\ \begin{vmatrix} 0 & \frac{1}{\gamma} & 1 \\ 1 & \beta & 1 \\ \frac{1}{1+\alpha} & 0 & 1 \end{vmatrix} \rightarrow \begin{vmatrix} 0 & \frac{m}{\gamma}+n & 1 \\ 1 & \beta & 1 \\ \frac{m}{m+\alpha} & \frac{n\alpha}{m+\alpha} & 1 \end{vmatrix} ;\ (f)\ \begin{vmatrix} 0 & \frac{1}{\gamma} & 1 \\ 1 & -\beta & 1 \\ \frac{1}{1-\alpha} & 0 & 1 \end{vmatrix} \rightarrow \begin{vmatrix} 0 & \frac{m}{\gamma}+n & 1 \\ 1 & -\beta & 1 \\ \frac{m}{m-\alpha} & \frac{-n\alpha}{m-\alpha} & 1 \end{vmatrix}$$

Type IV. $\alpha+\beta\gamma=0$, with two intersecting scales and a third straight scale.

The fundamental determinant here is

$$\begin{vmatrix} 0 & \dfrac{1}{\alpha+n} & 1 \\ \dfrac{\beta}{\beta+n} & \dfrac{1}{\beta+n} & 1 \\ \dfrac{1}{1+\gamma} & 0 & 1 \end{vmatrix} = 0 \ .$$

This form can replace Type III (c) when either or both of α and β pass through zero. The transformation here may be written

$$\begin{vmatrix} 0 & \dfrac{1}{\alpha+n} & 1 \\ \dfrac{\beta}{m\beta+n} & \dfrac{k\beta+1}{m\beta+n} & 1 \\ \dfrac{1}{m+\gamma} & \dfrac{k}{m+\gamma} & 1 \end{vmatrix} = 0 \ ;$$

where k removes any necessity to employ oblique axes, and m and n allow adjustment of the position of the γ and α scales with respect to each other, and also the origin.

Fourth Order Equations

The general form is

$$f_3(a_0f_1f_2 + a_1f_1 + a_2f_2 + a_3) + \phi_3(b_0f_1f_2 + b_1f_1 + b_2f_2 + b_3)$$
$$+ (c_0f_1f_2 + c_1f_1 + c_2f_2 + c_3) = 0 \ , \qquad (11)$$

where f_3 and ϕ_3 are not connected by a linear relation $\phi_3 = d_1f_3 + d_2$ (otherwise this general equation reduces to the third order). We may write the general equation

$$f_1f_2A_3 + f_1B_3 + f_2C_3 + D_3 = 0 \qquad (12)$$

where

$$A_3 = a_0f_3 + b_0\phi_3 + c_0 \ ; \qquad B_3 = a_1f_3 + b_1\phi_3 + c_1 \ ;$$
$$C_3 = a_2f_3 + b_2\phi_3 + c_2 \ ; \qquad D_3 = a_3f_3 + b_3\phi_3 + c_3 \ .$$

(i) When one of the expressions A_3, B_3, C_3, D_3 is identically zero.

(a) For $A_3 = 0$, the general form reduces to $\dfrac{D_3}{B_3} + f_1 + \dfrac{C_3}{B_3}f_2 = 0$ (13)

(b) For $B_3 = 0$, the general form reduces to $\dfrac{C_3}{A_3} + f_1 + \dfrac{D_3}{A_3} \cdot \left(\dfrac{1}{f_2}\right) = 0$ (14)

(c) For $C_3 = 0$, the general form reduces to $\dfrac{B_3}{A_3} + f_2 + \dfrac{D_3}{A_3}\left(\dfrac{1}{f_1}\right) = 0$ (15)

(d) For $D_3 = 0$, the general form reduces to $\dfrac{A_3}{C_3} + \dfrac{1}{f_1} + \dfrac{B_3}{C_3}\left(\dfrac{1}{f_2}\right) = 0$. (16)

(Should two of these expressions be zero, the case reduces to the third order).

Each of the above equations is of the canonical form $\alpha_1+\beta+\alpha_2\gamma=0$—($C$), which may be represented by a chart with two straight scales for the variables β and γ, and one curved scale for the variable $[\alpha_1\alpha_2]$, which is in each case the original variable $[f_3\phi_3]$. The transformations for this canonical form will be discussed at the conclusion of Case (ii) below.

When none of A_3, B_3, C_3, D_3 are zero, it is necessary to evaluate the four quantities

$$a = \begin{vmatrix} a_1 & b_1 & c_1 \\ a_2 & b_2 & c_2 \\ a_3 & b_3 & c_3 \end{vmatrix};\ b = \begin{vmatrix} a_0 & b_0 & c_0 \\ a_2 & b_2 & c_2 \\ a_3 & b_3 & c_3 \end{vmatrix};\ c = \begin{vmatrix} a_0 & b_0 & c_0 \\ a_1 & b_1 & c_1 \\ a_3 & b_3 & c_3 \end{vmatrix};\ d = \begin{vmatrix} a_0 & b_0 & c_0 \\ a_1 & b_1 & c_1 \\ a_2 & b_2 & c_2 \end{vmatrix}.$$

It will be shown that if $ad=bc$, a chart is possible composed of two straight scales for f_1 and f_2, and a curved scale for $[f_3\phi_3]$, but that if $ad\neq bc$ such a chart is impossible, the scales for f_1 and f_2 must be placed on a conic and the scale for $[f_3\phi_3]$ on a curve.

When none of A_3, B_3, C_3, D_3 are zero, it may be proved that one at least of a, b, c, d, differs from zero. It can also be shown that the identity

$$aA_3 - bB_3 + cC_3 - dD_3 \equiv 0 \tag{17}$$

is true. Thus no three of a, b, c, d may be zero.

Suppose $d\neq 0$. Then eliminating D_3 between Equations (12) and (17) we obtain $\left(f_1f_2+\frac{a}{d}\right)A_3+\left(f_1-\frac{b}{d}\right)B_3+\left(f_2+\frac{c}{d}\right)C_3=0.$

Substitute $X_1=f_1-\frac{b}{d}$; $X_2=f_2+\frac{c}{d}$, with the result

$$X_1X_2 + X_1\left(\frac{B_3}{A_3} - \frac{c}{d}\right) + X_2\left(\frac{C_3}{A_3} + \frac{b}{d}\right) + \frac{ad-bc}{d^2} = 0\ . \tag{18}$$

(*ii*) For $ad=bc$ the independent term in Equation (18) vanishes. Substitute back for X_1 and X_2 and divide through by $X_1X_2\left(\frac{C_3}{A_3}+\frac{b}{d}\right)$ and obtain

$$\frac{A_3}{dC_3+bA_3} + \frac{1}{df_1-b} + \frac{dB_3-cA_3}{dC_3+bA_3}\cdot\frac{1}{df_2+c} = 0\ . \tag{19d}$$

In a similar manner, by assuming each in turn of a, b, c, differing from zero, three other similar forms are obtained. In any example there is a choice of two of the four forms. The three remaining forms follow:—

For $a\neq 0$, $$\frac{D_3}{aB_3+cD_3} + \frac{f_1}{a-cf_1} + \frac{aC_3-bD_3}{aB_3+cD_3}\cdot\frac{f_2}{a+bf_2} = 0 \tag{19a}$$

For $b\neq 0$, $$\frac{C_3}{bA_3+dC_3} + \frac{f_1}{b-df_1} + \frac{bD_3-aC_3}{bA_3+dC_3}\cdot\frac{1}{a+bf_2} = 0 \tag{19b}$$

For $c\neq 0$, $$\frac{B_3}{aB_3+cD_3} + \frac{1}{cf_1-a} + \frac{cA_3-dB_3}{aB_3+cD_3}\cdot\frac{f_2}{c+df_2} = 0 \tag{19c}$$

The above four Equations (19) are of the type $\alpha_1+\beta+\alpha_2\gamma=0$ — (C) which will now be discussed.

Type V. $\alpha_1+\beta+\alpha_2\gamma=0$, with two parallel and one curved scale.

The fundamental determinants are

$$\begin{vmatrix} \frac{1}{1+\alpha_2} & \frac{-\alpha_1}{1+\alpha_2} & 1 \\ 0 & \gamma & 1 \\ 1 & \beta & 1 \end{vmatrix} = 0\ .$$

and

$$\begin{vmatrix} \frac{1}{1-\alpha_2} & \frac{\alpha_1}{1-\alpha_2} & 1 \\ 0 & \gamma & 1 \\ 1 & -\beta & 1 \end{vmatrix} = 0\ .$$

Note that the β scale has been reversed with respect to the γ scale.

These give rise to the transformations

$$\begin{vmatrix} \frac{1}{1+m\alpha_2} & \frac{n-m\alpha_1}{1+m\alpha_2} & 1 \\ 0 & \gamma & 1 \\ 1 & m\beta+n & 1 \end{vmatrix} = 0 \text{ and } \begin{vmatrix} \frac{1}{1-m\alpha_2} & \frac{n+m\alpha_1}{1-m\alpha_2} & 1 \\ 0 & \gamma & 1 \\ 1 & n-m\beta & 1 \end{vmatrix} = 0 \ .$$

The constant m magnifies and the constant n translates the β scale.

Type VI. $\alpha_1+\beta+\alpha_2\gamma=0$, with two intersecting straight scales and a curved scale.

The fundamental and transformed determinants here are

$$\begin{vmatrix} -\frac{\alpha_2}{\alpha_1} & -\frac{1}{\alpha_1} & 1 \\ 0 & \frac{1}{\beta} & 1 \\ \frac{1}{\gamma} & 0 & 1 \end{vmatrix} = 0 \text{ and } \begin{vmatrix} \frac{\alpha_2+k}{m+n\alpha_2-\alpha_1} & \frac{1}{m+n\alpha_2-\alpha_1} & 1 \\ \frac{k}{\beta+m} & \frac{1}{\beta+m} & 1 \\ \frac{1}{\gamma+n} & 0 & 1 \end{vmatrix} = 0 \ .$$

Remarks similar to those for Type II above apply here.

(*iii*) Fourth order equations requiring conic scales.

When $ad \neq bc$, the independent term of Equation (18) does not vanish. Writing $k=\frac{ad-bc}{d^2}$; $F_3=\frac{B_3}{A_3}-\frac{c}{d}$; $G_3=\frac{C_3}{A_3}+\frac{b}{d}$ and dividing through by X_2 this equation becomes

$$\left(X_1\frac{k}{X_2}\right)\frac{F_3}{K}+\left(X_1+\frac{k}{X_2}\right)+G_3=0 \ . \qquad (20)$$

This is symmetric in the functions X_1 and $\frac{k}{X_2}$ and may be expressed

$$\begin{vmatrix} -1 & G_3 & \frac{F_3}{k} \\ X_1 & X_1^2 & 1 \\ \frac{k}{X_2} & \left(\frac{k}{X_2}\right)^2 & 1 \end{vmatrix} = 0 \ . \qquad (21)$$

On expanding this, Equation (20) is obtained, with the added factor $\left(X_1-\frac{k}{X_2}\right)$. It is evident that in this form the alignment chart will have both the X_1 and the $\frac{k}{X_2}$ scales on the parabola $y=x^2$, and the $[f_3\phi_3]$ scale will lie on a curve defined by $x=-\frac{k}{F_3}$; $y=\frac{kG_3}{F_3}$. Since, however, the parabola is not suitable for practical use, two modifications of this determinant are used, one having a circle, and the other a hyperbola, take the place of the parabola.

To give the circular form we write $X_1=F_1$; $\frac{k}{X_2}=F_2$ for convenience and transform as follows:—

$$\text{Equation (21)}\rightarrow\begin{vmatrix} \frac{F_3}{k}+G_3 & -2 & \frac{F_3}{k}-G_3 \\ 1+F_1^2 & 2F_1 & 1-F_1^2 \\ 1+F_2^2 & 2F_2 & 1-F_2^2 \end{vmatrix} \rightarrow \begin{vmatrix} \frac{F_3-kG_3}{F_3+kG_3} & \frac{-2k}{F_3+kG_3} & 1 \\ \frac{1-F_1^2}{1+F_1^2} & \frac{2F_1}{1+F_1^2} & 1 \\ \frac{1-F_2^2}{1+F_2^2} & \frac{2F_2}{1+F_2^2} & 1 \end{vmatrix} = 0 \ .$$

The conic is here defined by $x=\dfrac{1-F^2}{1+F^2}$; $y=\dfrac{2F}{1+F^2}$; whence $x^2+y^2=1$, the unit circle. The hyperbolic form may be obtained in a similar manner

$$\begin{vmatrix} \dfrac{F_3+kG_3}{F_3-kG_3} & \dfrac{-2k}{F_3-kG_3} & 1 \\ \dfrac{1+F_1^2}{1-F_1^2} & \dfrac{2F_1}{1-F_1^2} & 1 \\ \dfrac{1+F_2^2}{1-F_2^2} & \dfrac{2F_2}{1-F_2^2} & 1 \end{vmatrix} = 0\,.$$

Here the conic is defined by $x=\dfrac{1+F^2}{1-F^2}$; $y=\dfrac{2F}{1-F^2}$, whence $x^2-y^2=1$, the rectangular hyperbola.

Using the circular form, the substitution $F_1=\tan\dfrac{\theta_1}{2}$ leads to the simple conditions $x=\cos\theta_1$; $y=\sin\theta_1$, which furnish an easy method of plotting values of the functions F_1 and F_2 on the circle. This may be done by drawing radii at angles θ_1 with the x axis, the points of intersection with the circle having the functional values $F_1=\tan\dfrac{\theta_1}{2}$. This has been done in Fig. 1, and values of F_1 (ϕ in the figure) at $\theta=0°$, $90°$, etc., are readily verified.

For the hyperbolic form the substitution $F_1=\tanh\dfrac{\theta_1}{2}$ is used, whence $x=\cosh\theta_1$; $y=\sinh\theta_1$. This form is also shown in Fig. 1.

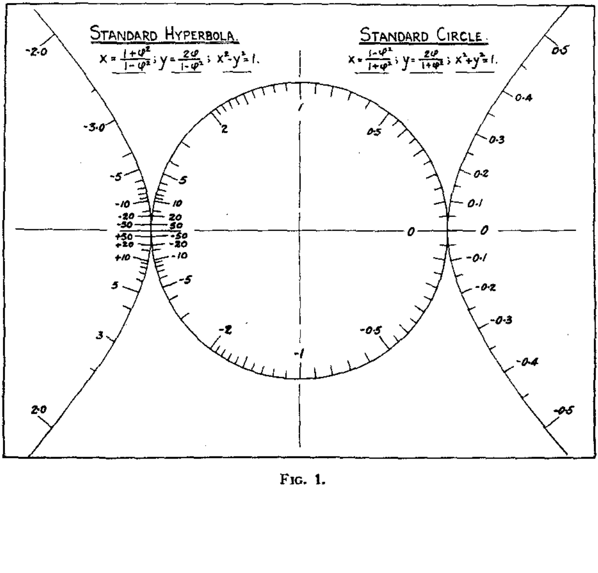


FIG. 1.

Of the two forms the circular is usually preferable because of its simple construction. However, in certain cases the third scale $[f_3\phi_3]$ may be found to lie at an inconvenient distance outside the circle. Whenever this is so, it will be found (no proof given here) that in the hyperbolic form the third scale will be between the two branches of the hyperbola.

The simplest method of studying the adjustment of scales is by using the parabolic form, and after the parabolic determinant has been transformed it may be put in either the circular or hyperbolic form.

We may write Equation (21) in the form

$$\begin{vmatrix} P_3 & Q_3 & R_3 \\ F_1 & F_1^2 & 1 \\ F_2 & F_2^2 & 1 \end{vmatrix} = 0 \tag{22}$$

where the subscripts designate the three functions. Equation (21) was obtained by assuming $d \neq 0$. There are three similar forms for each of a, b, c, not zero. In each of the four cases we may obtain, by substituting back, the equivalents of P_3, Q_3, R_3, F_1, and F_2 in terms of the original constants and functions of the general equation. These equivalents are as shown in Table I.

TABLE I

Elements of Parabolic Form (22)

$a \neq 0$	$b \neq 0$	$c \neq 0$	$d \neq 0$
$P_3 = -(ad-bc)D_3$	$(ad-bc)C_3$	$(ad-bc)B_3$	$(ad-bc)A_3$
$Q_3 = (ad-bc)(aC_3-bD_3)$	$(ad-bc)(aC_3-bD_3)$	$-(ad-bc)(cA_3-dB_3)$	$-(ad-bc)(bA_3+dC_3)$
$R_3 = aB_3+cD_3$	bA_3+dC_3	aB_3+cD_3	cA_3-dB_3
$F_1 = \dfrac{(ad-bc)f_1}{a-cf_1}$	$\dfrac{(ad-bc)f_1}{df_1-b}$	$\dfrac{ad-bc}{a-cf_1}$	df_1-b
$F_2 = \dfrac{a+bf_2}{f_2}$	$a+bf_2$	$\dfrac{c+df_2}{f_2}$	$\dfrac{ad-bc}{c+df_2}$

It is found to be impossible to shift the scales along the conic independently of each other; in other words, overlapping portions of the two scales will remain overlapping. Equal values of the functions F_1 and F_2 appear as coincident points on the conic, and remain so. It is possible, however, to shift these points around the conic and in this way translate and magnify the scales together. The transformation in parabolic form is

$$\begin{vmatrix} mP_3+nR_3 & m^2Q_3+2mnP_3+n^2R_3 & R_3 \\ mF_1+n & (mF_1+n)^2 & 1 \\ mF_2+n & (mF_2+n)^2 & 1 \end{vmatrix} = 0. \tag{23}$$

In the case of overlapping scales, very little may be done in a general way, the best plan being to try a few values of m and n and ascertain where the three scales lie. Each chart will be a study in itself.

In the case of non-overlapping scales, it may be found most suitable to arrange the conic scales symmetrically opposite each other. Suppose, for

instance, that we plot the extreme values of F_1 and of F_2 on the standard circle, denoting these end points of the scales by A, B, for F_1 and C, D, for F_2. Let us mark these so that in order around the circle they read $ABCD$. Then we must try to place A opposite C, and B opposite D.

To do this in our transformation, we have the conditions

$$(mA+n)(mC+n) = -1 \; ; \; (mB+n)(mD+n) = -1 .$$

Solving for m and n, this gives

$$m = \frac{A-B+C-D}{\sqrt{-(A-B)(B-C)(C-D)(D-A)}}$$

$$n = \frac{BD-AC}{\sqrt{-(A-B)(B-C)(C-D)(D-A)}} .$$

If it is found that this transformation places the third scale $[f_3\phi_3]$ at an inconvenient distance from the circle, the circular form of chart is still possible, as the $[f_3\phi_3]$ scale may be moved in closer to the circle. This is done by using a larger range of values for the variable which lies on the circle nearest the $[f_3\phi_3]$ scale. Thus we virtually reduce the working length of this circular scale, and the two conic scales will lie opposite each other, but one will be shorter than the other.

It is evident that in the determinant form the x column and the y column may be multiplied by any desired multiple, independently. This means that the abscissas and the ordinates in the chart may be plotted to any desired scale, not necessarily the same for both. In this way the conic scales may be plotted on an ellipse or on any desired hyperbola.

If such a procedure is intended in the case of the circular chart, and if it is found that the functional scales on the circle have their axis inclined at an angle α to the x axis, these scales should first be rotated until they are bisected by the x axis by using the substitutions $X = x \cos\alpha + y \sin\alpha$; $Y = y\cos\alpha - x\sin\alpha$, for all three scales in the circular determinant. Then all abscissas X and ordinates Y may be plotted according to independent scales, giving the elliptic form of chart. In the case of the hyperbolic chart this transformation will not be found necessary.

To summarize what has just been discussed:—

Type VII. Two scales on a circle and a curved scale.

The transformation of Equation (22) to Equation (23) with suitable values for m and n leads to the circular chart in which the functions f_1 and f_2 are represented by scales on the unit circle $\phi_1 = mF_1 + n$; $\phi_2 = mF_2 + n$, and the function $[f_3\phi_3]$ by the scale defined by $x = \dfrac{R_3(1-n^2) - 2mnP_3 - m^2Q_3}{R_3(1+n^2) + 2mnP_3 + m^2Q_3}$ and $y = \dfrac{2(mP_3 + nR_3)}{R_3(1+n^2) + 2mnP_3 + m^2Q_3}$; the co-ordinates of the f_1 and f_2 scales are $x = \dfrac{1-\phi_1^2}{1+\phi_1^2}$; $y = \dfrac{2\phi_1}{1+\phi_1^2}$; and $x = \dfrac{1-\phi_2^2}{1+\phi_2^2}$; $y = \dfrac{2\phi_2}{1+\phi_2^2}$.

Rotation by an angle α about the origin will be obtained by finding new co-ordinates in each of the three functions using the substitutions

$$X = x\cos\alpha + y\sin\alpha;\quad Y = y\cos\alpha - x\sin\alpha.$$

Type VIII. Two scales on a hyperbola and a curved scale.

In the case of the hyperbolic chart the three scales are finally given by

$$x = \frac{1+\phi_1^2}{1-\phi_1^2};\quad y = \frac{2\phi_1}{1-\phi_1^2}\ \text{for } f_1;\quad x = \frac{1+\phi_2^2}{1-\phi_2^2};\quad y = \frac{2\phi_2}{1-\phi_2^2}\ \text{for } f_2;$$

and

$$x = \frac{R_3(1+n^2)+2mnP_3+m^2Q_3}{R_3(1-n^2)-2mnP_3-m^2Q_3};\quad y = \frac{2(mP_3+nR_3)}{R_3(1-n^2)-2mnP_3-m^2Q_3}\ \text{for } [f_3\phi_3].$$

There follow some examples illustrating the preceding text.

Example 1. For a simply supported beam, as shown in Fig. 2, the shear at any section A is given by the formula

$$V = wl_1\left[\tfrac{1}{2}\left(\frac{x}{l_1}\right)^2 - \tfrac{1}{6}\frac{l_1}{l}\right] = Swl_1\ .$$

The chart at the left side has been drawn to solve for S. If $\frac{l_1}{l}$ is denoted by a, and $\frac{x}{l_1}$ by b, then $S = \frac{1}{2}b^2 - \frac{1}{6}a$. This is an equation of the third order already in the standard form $\alpha+\beta+\gamma=0$. Placing the a scale at the left

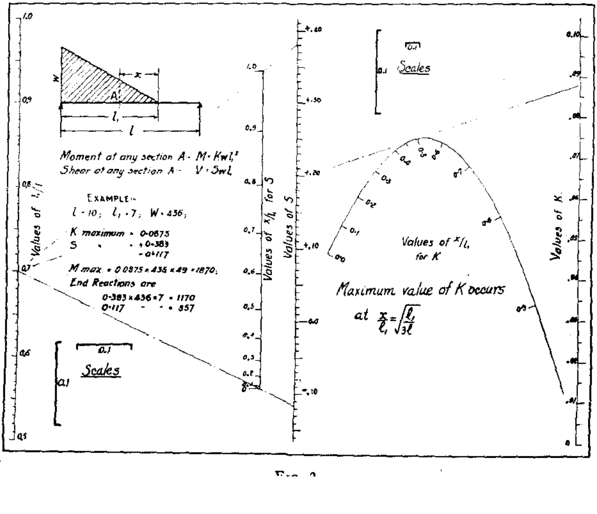


FIG. 2

and the S scale at the right in a chart of Type I will require that $\alpha = \frac{1}{6}a$; $\gamma = S$; $\beta = -\frac{1}{2}b^2$.

Using a range for a from 0.5 to 1.0, it is evident from the equation that the extreme values for S are $+\frac{5}{12}$ at the left support when $\frac{l_1}{l} = 0.5$, and $-\frac{1}{6}$ at the right support when $\frac{l_1}{l} = 1.0$. For the α and γ scales to lie opposite each other we must have

$$\alpha_1 = m\gamma_1 + n \ , \quad i.e., \quad \frac{0.5}{6} = m(-\tfrac{1}{6}) + n$$

$$\alpha_2 = m\gamma_2 + n \ , \quad i.e., \quad \frac{1.0}{6} = m(\tfrac{5}{12}) + n \ .$$

Solving for m and n, $m = \frac{1}{7}$; $n = \frac{3}{28}$, and the final determinant, after multiplying all elements in the centre column by 6, becomes

$$\begin{vmatrix} 0 & a & 1 \\ 1.75 & 0.375\,b^2 + 0.5625 & 1 \\ 2 & \frac{6}{7}S + \frac{9}{14} & 1 \end{vmatrix} = 0 \ .$$

The resulting chart thus has the a or $\frac{l_1}{l}$ scale defined by $x = 0$; $y = \frac{l_1}{l}$; the b or $\frac{x}{l_1}$ scale defined by $x = 1.75$; $y = \frac{3}{8}\left(\frac{x}{l_1}\right)^2 + \frac{9}{16}$; and the S scale defined by $x = 2$; $y = \frac{6}{7}S + \frac{9}{14}$.

If it were desired to construct the chart with the S scale in the centre, then we must put $\alpha = \frac{1}{6}a$; $\gamma = -\frac{1}{2}b^2$; $\beta = S$. In this case we must equate extreme values of α and γ in the transformation, so that $\frac{1}{6}(0.5) = m(-\frac{1}{2} \times 1^2) + n$; $\frac{1}{6}(1.0) = m(0) + n$, whence $m = \frac{1}{6}$; $n = 1$. In this chart $a = 0.5$ lies opposite $b = 1$, and $a = 1.0$ lies opposite $b = 0$. Note that the b scale is reversed when the S scale is placed in the centre. The determinant form here is

$$\begin{vmatrix} 0 & a & 1 \\ \frac{12}{7} & \frac{6}{7}(1 - S) & 1 \\ 2 & 1 - \frac{1}{2}b^2 & 1 \end{vmatrix} = 0 \ .$$

Example 2. For the same beam of Fig. 2 the bending moment at any section A is given by $M = wl_1^2\left[\frac{x + l - l_1}{6l} - \frac{x^3}{6l_1^3}\right] = Kwl_1^2$ and the chart at the right side solves for K.

Denoting as before $\frac{l_1}{l}$ by a and $\frac{x}{l_1}$ by b the formula becomes $K = \frac{1}{6}(ab + 1 - a - b^3)$. This is seen to be an equation of the fourth order and without taking the trouble to go through all the steps given in the text, we can by trial put it in the form $\alpha_1 + \beta + \alpha_2\gamma = 0$ as follows:—

$6K - 1 = ab - a - b^3 = a(b - 1) - b^3$, or $b^3 + (6K - 1) + (1 - b)a = 0$, and we may substitute $\alpha_1 = b^3$; $\alpha_2 = (1 - b)$; $\beta = 6K - 1$; $\gamma = a$.

We have chosen in the chart to place the γ and β scales on the outside and the curved $[\alpha_1\alpha_2]$ scale in between. We must therefore choose the first

of the two transformations of Type V, since the range for $\alpha_2 = (1-b)$ is 0 to 1 and hence $\frac{1}{1+\alpha_2}$ ranges between 1 and $\frac{1}{2}$.

The extreme limits of a are, as before, 0.5 and 1.0. The maximum value of K is found to occur at $a = 0.5$ and $b = \sqrt{\frac{1}{6}}$, giving K maximum $= 0.106$. (The proof of this involves an interesting study of the expression $ab+1-a-b^3$ which will not be given in detail here.) For γ and β scales opposite each other, extreme values are equated, giving

$$\gamma_1 = m\beta_1 + n \quad \text{or} \quad 0.5 = m(-1) + n$$
$$\gamma_2 = m\beta_2 + n \quad \text{or} \quad 1.0 = m(6+0.106-1) + n \ .$$

Hence $m = 0.786$ and $n = 1.286$, and the final determinant form is

$$\begin{vmatrix} \dfrac{1}{1.786 - 0.786b} & \dfrac{1.286 - 0.786b^3}{1.786 - 0.786b} & 1 \\ 0 & a & 1 \\ 1 & 0.5 + 4.716K & 1 \end{vmatrix} = 0 \ .$$

If it were desired to place the K scale in the centre, the second of the two transformations of Type V should be used. Here it is necessary to arrange the straight a scale opposite the curved b scale, which is rather difficult. However, we may first place $a = 1.0$ opposite $b = 1.0$ and then arrange the constants m and n so that the lowest point of the curved b scale will be opposite $a = 0.5$. The first condition, using $\gamma = \frac{n+m\alpha_1}{1-m\alpha_2}$, gives $1.0 = \frac{n+m}{1-0} = n+m$. The second condition is that the minimum value of $\frac{n+mb^3}{1-m(1-b)}$ shall be 0.5, for b lying between 0 and 1.

Substituting $n = 1-m$ this gives us $\frac{1+m(b^3-1)}{1+m(b-1)} \geqq 0.5$, which may be written $\frac{1-m}{m} \geqq b(1-2b^2)$ or that $\frac{1-m}{m}$ shall equal the maximum value of $b(1-2b^2)$.

By differentiation the maximum of this expression occurs at $b = \sqrt{\frac{1}{6}}$, whence $\frac{1-m}{m} = \sqrt{\frac{1}{6}}(1-\frac{1}{3})$ and $m = 0.786$. Hence $n = 0.214$.

Another method of determining m and n would have been to place the greatest value of K (*i.e.*, 0.106) opposite the value of $a = 0.5$. Thus

$$\gamma_1 = n - m\beta_1 \text{ or } 0.5 = n - m(0.636-1)\ ; \quad \gamma_2 = n - m\beta_2 \text{ or } 1 = n - m(-1);$$

giving $m = 0.786$; $n = 0.214$ as before.

The final determinant form would be

$$\begin{vmatrix} \dfrac{1}{0.214 + 0.786b} & \dfrac{0.214 + 0.786b^3}{0.214 + 0.786b} & 1 \\ 0 & a & 1 \\ 1 & 1 - 4.716K & 1 \end{vmatrix} = 0 \ .$$

Example 3. For lack of a more common equation to illustrate Types VII and VIII, the historic formula of Massau's is chosen

$$(1+L)H^2 - LH(1+p) - \tfrac{1}{3}(1-L)(1+2p) = 0 \ .$$

Putting this in the general form (Equation 11), it becomes

$$3H^2(0+L+0+1) - 3H(Lp+L+0+0) + (2Lp+L-2p-1) = 0 \ .$$

On inspection none of the expressions A_3, B_3, C_3, D_3 are identically zero. We must therefore evaluate

$$a=\begin{vmatrix} 1 & 1 & 1 \\ 0 & 0 & -2 \\ 1 & 0 & -1 \end{vmatrix}=-2;\ b=\begin{vmatrix} 0 & 1 & 2 \\ 0 & 0 & -2 \\ 1 & 0 & -1 \end{vmatrix}=-2;\ c=\begin{vmatrix} 0 & 1 & 2 \\ 1 & 1 & 1 \\ 1 & 0 & -1 \end{vmatrix}=0;\ d=\begin{vmatrix} 0 & 1 & 2 \\ 1 & 1 & 1 \\ 0 & 0 & -2 \end{vmatrix}=2.$$

Hence $ad-bc=-4$, and a conic chart is necessary.

Looking over the forms of Equation (22) in Table I we choose the one for $a\neq 0$, giving

$$\begin{aligned} P_3 &= 4D_3 = 4(3H^2-1) \\ Q_3 &= -4(-2C_3+2D_3) = -8(3H^2+1) \\ R_3 &= -2B_3 = -2(3H^2-3H+1) \\ F_1 &= \frac{-4f_1}{-2} = 2L \\ F_2 &= \frac{-2-2f_2}{f_2} = -2\left(\frac{1+p}{p}\right) \ . \end{aligned}$$

Using ranges of values of L from 0.5 to 1.0 and of p from 0.5 to 1.0, the corresponding limits of F_1 are 1 and 2, and F_2 ranges from -6 to -4. The four limiting points on the circle which are to be arranged symmetrically are $A=1$; $B=2$; $C=-6$; $D=-4$.

Before proceeding it may be as well to check up on the position of the H scale. It is found to have a range of values from $H=0.75$ to 1.0 and in the circular chart (before transformation) $x=\frac{R_3-Q_3}{R_3+Q_3}$; $y=\frac{2P_3}{R_3+Q_3}$ tells us that x varies from $-\frac{165}{179}$ to $-\frac{15}{17}$ and y from $-\frac{44}{179}$ to $-\frac{8}{17}$. The H scale therefore lies within the circle.

Proceeding with the transformation for symmetry of the conic scales,

$$m=\frac{A-B+C-D}{\sqrt{\text{etc.}}}=-\frac{3}{\sqrt{80}}\ ; \quad n=\frac{BD-AC}{\sqrt{\text{etc.}}}=-\frac{2}{\sqrt{80}} \ .$$

Substituting these values in our summary for Type VII we find the ϕ_1 or L scale located by

$$\begin{aligned} x &= \frac{1-(mF_1+n)^2}{1+(mF_1+n)^2} = \frac{19-6L-9L^2}{21+6L+9L^2} \\ y &= \frac{2(mF_1+n)}{1+(mF_1+n)^2} = \frac{-20(3L+1)}{\sqrt{5}(21+6L+9L^2)} \ . \end{aligned}$$

The ϕ_2 or p scale is located by

$$x=\frac{16p^2-12p-9}{24p^2+12p+9}\ ; \quad y=\frac{20p(2p+3)}{\sqrt{5}\,(24p^2+12p+9)}\ ;$$

and the $[f_3\phi_3]$ scale located by

$$x=\frac{4.8H^2-5.7H+0.4}{7.2H^2-6.3H+3.6}\ ; \quad y=\frac{2(6H^2+3H-4)}{0.9\sqrt{5}\,(8H^2-7H+4)} \ .$$

To determine the rotation of the scales, the range of values of $\phi_1 = mF_1 + n$ is found to be $-\frac{2.5}{\sqrt{20}}$ to $-\frac{4}{\sqrt{20}}$, and of $\phi_2 = mF_2 + n$ from $\frac{8}{\sqrt{20}}$ to $\frac{5}{\sqrt{20}}$. Using $\tan\frac{\theta}{2} = \phi$, this gives a range for θ_2 from 121° 35′ to 96° 23′, so that the angle of rotation $\alpha = 108° 59'$, and the required substitutions are

$$X = x\cos\alpha + y\sin\alpha = -.3253x + .9456y$$
$$Y = y\cos\alpha - x\sin\alpha = -.9456x - .3253y$$

The result is the chart shown in Fig. 3.

For the hyperbolic chart this last rotation is not necessary and the three scales are given by

$$x = \frac{21 + 6L + 9L^2}{19 - 6L - 9L^2}; \qquad y = \frac{-20(3L+1)}{\sqrt{5}(19 - 6L - 9L^2)} \text{ for } \phi_1 \text{ or } L$$
$$x = \frac{24p^2 + 12p + 9}{16p^2 - 12p - 9}; \qquad y = \frac{20p(2p+3)}{\sqrt{5}(16p^2 - 12p - 9)} \text{ for } \phi_2 \text{ or } p$$
$$x = \frac{7.2H^2 - 6.3H + 3.6}{4.8H^2 - 5.7H + 0.4}; \qquad y = \frac{2(6H^2 + 3H - 4)}{\sqrt{5}(4.8H^2 - 5.7H + 0.4)} \text{ for } [f_3\phi_3] \text{ or } H.$$

This chart is shown in Fig. 4.

Conic Charts for Third Order Equations

As mentioned before, all third order equations may be represented by nomograms in which two of the scales lie on a single conic and the third scale on a straight line. We have already shown that all such equations may be expressed in one or other of the canonical forms (*A*) $\alpha+\beta+\gamma=0$, (*B*) $\alpha+\beta\gamma=0$. However, in Class (*b*) when $P^2 + B_1B_2B_3 < 0$, this reduction was possible only by the use of inverse tangents, and in this study of conic charts it is desirable to treat this Class (*b*) separately from the other two, and it will be called the transcendental type.

From Equation (5), which may be written $\tan^{-1}\alpha + \tan^{-1}\beta + \tan^{-1}\gamma = 0$, we have directly that $\alpha\beta\gamma - (\alpha+\beta+\gamma) = 0$ — (*D*) our third canonical form for third order equations. Here $\alpha = \frac{B_1X_1 + P}{R_1}$; $\beta = \frac{B_2X_2 + P}{R_1}$; $\gamma = \frac{R_1X_3}{PX_3 - B_1B_2}$; but of course owing to symmetry the functions may be interchanged.

A study of Equations (20) and (21) shows that the symmetric equation

$$f_1f_2A_3 + (f_1+f_2)B_3 + C_3 = 0 \tag{24}$$

leads to the parabolic determinant

$$\begin{vmatrix} -B_3 & C_3 & A_3 \\ f_1 & f_1^2 & 1 \\ f_2 & f_2^2 & 1 \end{vmatrix} = 0\,. \tag{25}$$

For fourth order equations, the scale for $[f_3\phi_3]$ was curved because the functions in the top row of the determinant were not linearly connected. Here in the third order equations, owing to the linear relation between A_3, B_3 and C_3, this scale will be found to lie on a straight line.

(*1*). $\alpha+\beta+\gamma=0$ — (*C*). This may be put into the form of Equation (24)

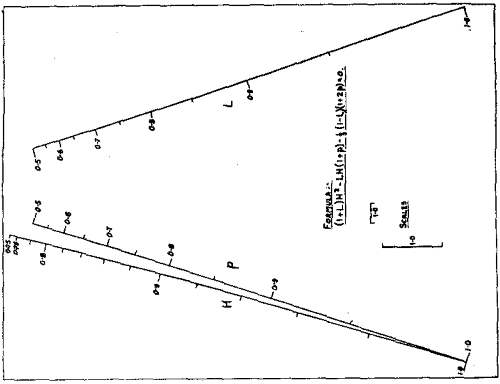


FIG. 4.

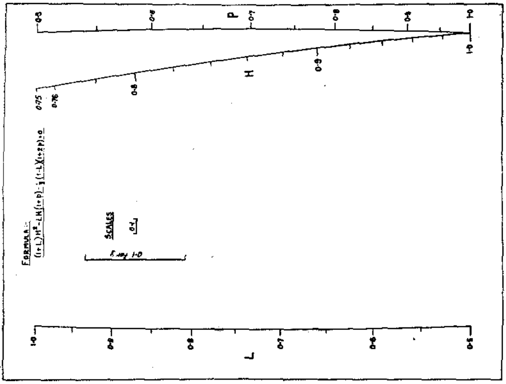


FIG. 3.

by making $A_3=0$, $B_3=1$, and $C_3=f_3$. In this case therefore Determinant (25) becomes

$$\begin{vmatrix} -1 & f_3 & 0 \\ f_1 & f_1^2 & 1 \\ f_2 & f_2^2 & 1 \end{vmatrix} = 0 \ .$$

representing $f_1+f_2+f_3=0$. Write $f_1=\beta$; $f_2=\gamma$; $f_3=\alpha$.

To shift the scales with respect to one another we may write the given canonical form $(m\beta+k)+(m\gamma+l)+(m\alpha-k-l)=0$ and the resulting chart will be indicated by

$$\begin{vmatrix} -1 & m\alpha-k-l & 0 \\ m\beta+k & (m\beta+k)^2 & 1 \\ m\gamma+l & (m\gamma+l)^2 & 1 \end{vmatrix} = 0 \ .$$

We have here chosen β and γ for the scales on the conic, and α on the straight line; m, k and l should be chosen so as to place the scales advantageously.

If the circular chart is chosen, the α scale is defined by $x=-1$; $y=\dfrac{-2}{m\alpha-k-l}$, which is tangent to the circle at $x=-1$.

It will be most convenient therefore to have the β and γ scales bisected by the x axis, *i.e.*, one passing through $\phi=0$ and the other through $\phi=\infty$.

In each particular example the ranges of the variables will determine the best adjustment, but the following pointers may not be amiss.

(*a*) If only one of the variables β,γ passes through the value ∞, the form given above is suitable as it stands.

(*b*) If neither β nor γ passes through the value ∞ the original equation should be written $(\alpha-p)+\beta+(\gamma+p)=0$, where p is so chosen as to make one of the conic scales pass through the value zero, and also ensure that the scales do not overlap.

The reciprocal form

$$\begin{vmatrix} -1 & 0 & \alpha-p \\ \dfrac{1}{\beta} & \left(\dfrac{1}{\beta}\right)^2 & 1 \\ \dfrac{1}{\gamma+p} & \left(\dfrac{1}{\gamma+p}\right)^2 & 1 \end{vmatrix} = 0$$

will then have the β and γ scales with one passing through $\phi=\infty$ and the other not. The final transformation for this is

$$\begin{vmatrix} k\alpha_1-m & k^2\alpha_1-2km & \alpha_1 \\ m\beta_1+k & (m\beta_1+k)^2 & 1 \\ m\gamma_1+k & (m\gamma_1+k)^2 & 1 \end{vmatrix} = 0$$

where we have written $\alpha_1=\alpha-p$; $\beta_1=\dfrac{1}{\beta}$; $\gamma_1=\dfrac{1}{\gamma+p}$.

Here m and k may be chosen to adjust the scales symmetrically.

(*c*) When both β and γ pass through the value infinity, the procedure of Case (*b*) may be followed. First, p is so chosen in $(\alpha-p)+\beta+(\gamma+p)=0$ that one of the conic scales does not pass through $\phi=0$ and the other does. Then in the reciprocal form both conic scales will pass through zero and only one through $\phi=\infty$. No values of m and k can prevent the scales from over-

lapping so that no suitable symmetric form is possible, even with the hyperbola. It is advisable in these rare cases either to construct two or more charts, each covering part of the range of values, or to replace one of the conic scales by α.

(*2*). $\alpha+\beta\gamma=0$ — (*B*). Substituting in Equation (24) $f_1=\beta$; $f_2=\gamma$; $A_3=1$; $B_3=0$, $C_3=\alpha$ gives the canonical form. Determinant (25) then becomes

$$\begin{vmatrix} 0 & \alpha & 1 \\ \beta & \beta^2 & 1 \\ \gamma & \gamma^2 & 1 \end{vmatrix} = 0 \, .$$

It is evident from the symmetry of the alternative form $\frac{1}{\alpha} \cdot \beta \cdot \gamma = -1$ that any two functions may be chosen for the conic scales. To effect the shifting of the scales with respect to one another the transformation

$$\begin{vmatrix} k & mn\alpha + k^2 & 1 \\ m\beta + k & (m\beta + k)^2 & 1 \\ n\gamma + k & (n\gamma + k)^2 & 1 \end{vmatrix} = 0$$

may be used.

In the circular chart the α scale is found to cut the circle. (In the fundamental form this α scale lies on the x axis.) Hence the circular form of chart will be most suitable, as in the hyperbolic form the α scale will lie to one side and not between the two branches. The most suitable method of determining m, n and k seems to be by trial. Note that a multiple m or n greater than unity shifts the scales towards the left of the circle, and a constant k positive moves the scales in a counterclockwise direction. As in Case (1, *c*) above, it is impossible to obtain suitable transformations when the β and γ scales cover very wide ranges of value (*e.g.*, through infinity). Such conditions seldom arise when accurate results are required, and charts covering such wide ranges are useful for general study and comparison only. Either the transformation given above, or its reciprocal,

$$\begin{vmatrix} k & 1 & mn\alpha + k^2 \\ \dfrac{1}{m\beta + k} & \left(\dfrac{1}{m\beta + k}\right)^2 & 1 \\ \dfrac{1}{n\gamma + k} & \left(\dfrac{1}{n\gamma + k}\right)^2 & 1 \end{vmatrix} = 0$$

should be sufficient to cover all practical cases. Otherwise, two or more charts should be used, or else one of the conic scales should be replaced by α.

(*3*). The transcendental type $\alpha\beta\gamma-(\alpha+\beta+\gamma)=0$ — (*D*).

For this case we substitute in Equation (24) $f_1=\beta$; $f_2=\gamma$; $A_3=\alpha$; $B_3=-1$; $C_3=-\alpha$. Hence Determinant (25) becomes

$$\begin{vmatrix} 1 & -\alpha & \alpha \\ \beta & \beta^2 & 1 \\ \gamma & \gamma^2 & 1 \end{vmatrix} = 0 \, .$$

To effect the transformation here both conic scales are first moved together, so that the β scale will be in its final position.

Thus

$$\begin{vmatrix} m + n\alpha & -m^2\alpha + 2mn + n^2\alpha & \alpha \\ m\beta + n & (m\beta + n)^2 & 1 \\ m\gamma + n & (m\gamma + n)^2 & 1 \end{vmatrix} = 0, \text{ which we write } \begin{vmatrix} -G_1 & H_1 & F_1 \\ \beta_1 & \beta_1^2 & 1 \\ \gamma_1 & \gamma_1^2 & 1 \end{vmatrix} = 0 \, .$$

Assume now that it is possible to use a transformation $\gamma_2 = \dfrac{p+q\gamma_1}{r+s\gamma_1}$, where p, q, r, s are constants to be determined, so as to satisfy the condition of symmetry in β_1 and γ_2.

The assumption may be written $\gamma_1 = -\dfrac{p-r\gamma_2}{q-s\gamma_2}$. On substituting this in the equation $\beta_1\gamma_1F_1+(\beta_1+\gamma_1)G_1+H_1=0$ we obtain

$$\beta_1\gamma_2(rF_1-sG_1)+\beta_1(qG_1-pF_1)+\gamma_2(rG_1-sH_1)+(qH_1-pG_1)=0 \tag{26}$$

For symmetry in β_1 and γ_2 the condition is $qG_1-pF_1=rG_1-sH_1$ which reduces to $(q-r)(m+n\alpha)+p\alpha-s(-m^2\alpha+2mn+n^2\alpha)\equiv 0$.

Hence $(q-r)n+p+(m^2-n^2)s=0$; $q-r-2ns=0$.

These two conditions reduce to $p=-(m^2+n^2)s$ and $q=r+2ns$.

Substitute now for p and q, F_1, G_1, and H_1 in Equation (26), and put $R=r+ns$; $S=ms$ and the symmetric form $\beta_1\gamma_2[R\alpha+S]+(\beta_1+\gamma_2)[(mS-nR)\alpha-(mR+nS)]+[(n^2R-m^2R-2mnS)\alpha+(n^2S-m^2S+2mnR)]=0$ results.

Making $S=$ unity does not interfere with the generality, and we have the final parabolic form

$$\begin{vmatrix} (nR-m)\alpha+(mR+n) & [(n^2-m^2)R-2mn]\alpha+2mnR+n^2-m^2 & R\alpha+1 \\ m\beta+n & (m\beta+n)^2 & 1 \\ \dfrac{(mR+n)\gamma+(nR-m)}{R+\gamma} & (\gamma\ \text{function})^2 & 1 \end{vmatrix}=0. \tag{27}$$

Here m and n may be chosen first, then R, to give suitable positions. Note that in this case the α scale lies outside the circle and between the branches of the hyperbola.

The transformations given in Cases (1) and (2) are particular cases given by the method which has just been used in Case (3), but owing to the simple nature of the canonical forms, it was possible to find them by much simpler routes. The various cases of the general third order equation (seven of them) may also be studied by this method, but such a procedure proved tedious and complicated. The study given above, of the three canonical forms, to which they can all be reduced, has been found preferable.

Example 4. Tan $(P+Q)=\dfrac{\tan P+\tan Q}{1-\tan P\tan Q}$ illustrates Case (3) above.

This may be written $-\tan(P+Q)\tan P.\tan Q-(\tan P+\tan Q-\tan\overline{P+Q})=0$, evidently of canonical type (D).

If we use the circular chart (Fig. 5) and assume a range of values for both tan P and tan Q from 0 to 1, and place the tan P scale on the right, extending from $\phi=-\frac{1}{4}$ to $\phi=+\frac{1}{4}$, we have the conditions (see Equation (27)) $m.0+n=-\frac{1}{4}$; $m.1+n=+\frac{1}{4}$, whence $m=\frac{1}{2}$; $n=-\frac{1}{4}$.

Choosing now a symmetrical position for the Q scale at the left we must use the condition

$$\frac{(mR+n)0+(nR-m)}{R+0}=-\frac{(mR+n)1+(nR-m)}{R+1}.$$

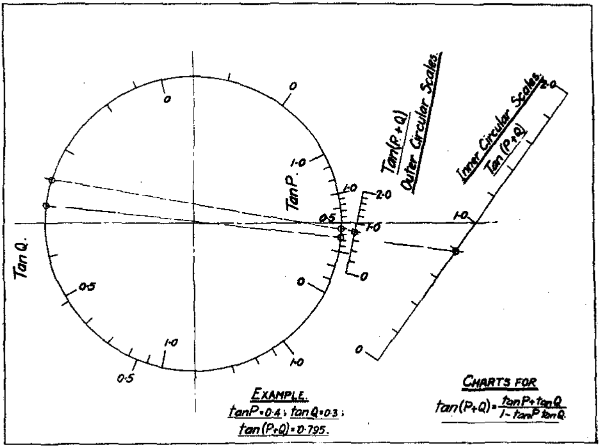
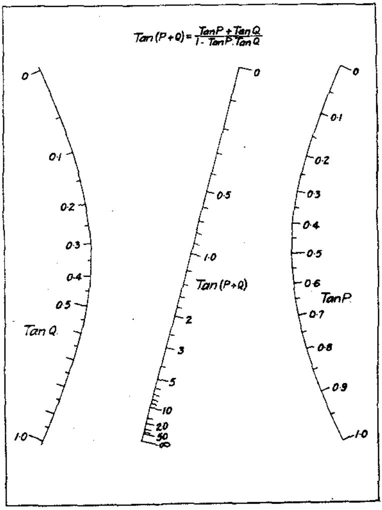

Tan(P+Q) Outer Circular Scales.
Inner Circular Scales Tan (P+Q)
Tan P.
Tan Q.
EXAMPLE.
tanP=0·4; tanQ=0·3;
tan(P+Q)=0·795.
CHARTS FOR
tan(P+Q)=tanP+tanQ/1-tanP tanQ

FIG. 5.


Tan(P+Q)=TanP+TanQ/1-TanP.TanQ
Tan Q
Tan (P+Q)
Tan P

Clearing and giving m and n their values, this makes $R=-\frac{1}{3}$. Equation (27) then reduces to

$$\begin{vmatrix} \frac{5}{4}(1-\tan\overline{P+Q}) & \frac{5}{16}(3\tan\overline{P+Q}+1) & -\tan(P+Q)-3 \\ \frac{1}{2}\tan P-\frac{1}{4} & \text{Square} & 1 \\ \frac{5}{8}\frac{\tan Q+1}{1-3\tan Q} & \text{Square} & 1 \end{vmatrix}=0$$

from which we obtain the circular chart with the two scales printed on the inside of the perimeter. The $\tan(P+Q)$ scale is plotted as $x=\dfrac{31\tan(P+Q)+53}{\tan(P+Q)+43}$; $y=\dfrac{40\tan(P+Q)-40}{\tan(P+Q)+43}$; and is the outer of the two straight scales.

The use of a standard circular chart drawn the same size as the final chart and on which is marked accurately a complete ϕ scale, will be found to aid the plotting. This standard chart may be placed under a tracing cloth and the final chart plotted on the tracing.

The second set of scales shown in the figure may be obtained in a similar manner, but a much simpler way is to use the parabolic form of the first chart, multiplying the ϕ or x column by $\frac{2}{5}$ and the ϕ^2 or y column by $\frac{4}{25}$. In this way the tan P scale is reduced and the tan Q scale enlarged, the straight scale being brought in closer.

Fig. 6 illustrates the hyperbolic form for this equation, the straight scale lying between the branches of the conic. The inclination of this scale from the vertical is due to a slight lack of symmetry between the two conic scales.

Cubic Charts for Third Order Equations

Although this type of chart is not suitable for practical purposes, it is included here for the sake of completeness. Just as the conic charts are based on the idea of symmetry of two of the variables, so the cubic charts are based on symmetry of all three variables, the three scales appearing on a single (cubic) curve.

All third order equations may be put in the form

$$\phi_1\phi_2\phi_3+A(\phi_1\phi_2+\phi_2\phi_3+\phi_3\phi_1)+B(\phi_1+\phi_2+\phi_3)+C=0 \qquad (28)$$

and this leads to the determinant form

$$\begin{vmatrix} \phi_1+A & \phi_1^2-B & \phi_1^3+C \\ \phi_2+A & \phi_2^2-B & \phi_2^3+C \\ \phi_3+A & \phi_3^2-B & \phi_3^3+C \end{vmatrix}=0\ . \qquad (29)$$

Expanding Equation (29) gives Equation (28) with three factors $(\phi_1-\phi_2)(\phi_2-\phi_3)(\phi_3-\phi_1)$. This determinant evidently gives a chart with all three variables on the same curve. If the first column is made unity, the curve is defined by $x=\dfrac{\phi^2-B}{\phi+A}$; $y=\dfrac{\phi^3+C}{\phi+A}$. On eliminating ϕ the equation $(C-A^3)x^3+(B-A^2)(x^2-y)y+(C-AB)(3Ax-y+3B)x-2(AC-B^2)y$

Similar equations result from making the other columns unity.

Let us now study each of the three canonical forms.

(*i*). $\phi_1+\phi_2+\phi_3=0$. For this form we substitute in Equation (28) $A=C=0$; $B=\infty$, giving the determinant

$$\begin{vmatrix} \phi_1 & 1 & \phi_1^3 \\ \phi_2 & 1 & \phi_2^3 \\ \phi_3 & 1 & \phi_3^3 \end{vmatrix} = 0 \;.$$

Making in turn each of the three columns unity here we obtain the three cubic charts

$$(a)\quad x=\frac{1}{\phi}\;;\quad y=\phi^2\;;\quad x^2y=1\;;$$

$$(b)\quad x=\phi\;;\quad y=\phi^3\;;\quad x^3=y\;;$$

$$(c)\quad x=\frac{1}{\phi^2}\;;\quad y=\frac{1}{\phi^3}\;;\quad x^3=y^2\;.$$

For these fundamental forms see Fig. 7 (i).

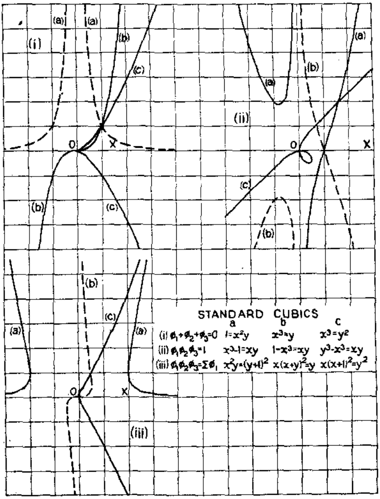


FIG. 7.

It is evident that the scales may be shifted with respect to one another by re-writing the canonical form $(m\phi_1+k)+(m\phi_2+l)+(m\phi_3-k-l)=0$.

(*ii*). $\phi_1\phi_2\phi_3=1$, which corresponds to $\alpha+\beta\gamma=0$.

Here substitute in Equation (28) $A=B=0$; $C=-1$ and the determinant becomes

$$\begin{vmatrix} \phi_1 & \phi_1^2 & \phi_1^3-1 \\ \phi_2 & \phi_2^2 & \phi_2^3-1 \\ \phi_3 & \phi_3^2 & \phi_3^3-1 \end{vmatrix} = 0 \ .$$

By making in turn each of the three columns unity the three cubics are found to be

$$(a)\quad x=\phi\ ; \qquad y=\frac{\phi^3-1}{\phi}\ ; \qquad x^3-1=xy$$

$$(b)\quad x=\frac{1}{\phi}\ ; \qquad y=\frac{\phi^3-1}{\phi^2}\ ; \qquad 1-x^3=xy$$

$$(c)\quad x=\frac{\phi}{\phi^3-1}\ ; \qquad y=\frac{\phi^2}{\phi^3-1}\ ; \qquad y^3-x^3=xy\ .$$

See Fig. 7 (ii). In this case the scales may be shifted by re-writing the given form $(l\phi_1)\ .\ (m\phi_2)\left(\frac{\phi_3}{lm}\right)=1$. Other transformations are possible but it is not worth while to study them here.

(*iii*). $\phi_1\phi_2\phi_3-(\phi_1+\phi_2+\phi_3)=0$. In Equation (28) put $A=C=0$; $B=-1$, giving the determinant

$$\begin{vmatrix} \phi_1 & \phi_1^2+1 & \phi_1^3 \\ \phi_2 & \phi_2^2+1 & \phi_2^3 \\ \phi_3 & \phi_3^2+1 & \phi_3^3 \end{vmatrix} = 0 \ .$$

Making in turn each of the three columns unity, we obtain

$$(a)\quad x=\frac{\phi^2+1}{\phi}\ ; \qquad y=\phi^2\ ; \qquad x^2y=(y+1)^2\ ;$$

$$(b)\quad x=\frac{\phi}{\phi^2+1}\ ; \qquad y=\frac{\phi^3}{\phi^2+1}\ ; \qquad x(x+y)^2=y\ ;$$

$$(c)\quad x=\frac{1}{\phi^2}\ ; \qquad y=\frac{\phi^2+1}{\phi^3}\ ; \qquad x(x+1)^2=y^2\ .$$

See Fig. 7 (iii). The transformation for shifting is more difficult here. One good method, which will be used, is to write the equation in the form $\tan^{-1}\phi_1+\tan^{-1}\phi_2+\tan^{-1}\phi_3=0$, whence $(\tan^{-1}\phi_1+\tan^{-1}l)+(\tan^{-1}\phi_2+\tan^{-1}m)+(\tan^{-1}\phi_3-\tan^{-1}l-\tan^{-1}m)=0$ is obtained directly.

This reduces to $\tan^{-1}\frac{\phi_1+l}{1-l\phi_1}+\tan^{-1}\frac{\phi_2+m}{1-m\phi_2}+\tan^{-1}\frac{(1-lm)\phi_3-(l+m)}{(1-lm)+(l+m)\phi_3}=0$,

which may be written $F_1F_2F_3=F_1+F_2+F_3$, where

$$F_1=\frac{\phi_1+l}{1-l\phi_1}\ ; \quad F_2=\frac{\phi_2+m}{1-m\phi_2}\ ; \quad F_3=\frac{(1-lm)\phi_3-(l+m)}{(1-lm)+(l+m)\phi_3}\ .$$

Example 5. We use once more the formula for $\tan(\alpha+\beta)$, choosing for its representation the cubic (iii b), $x(x+y)^2=y$. We put $\phi_1=\tan\alpha$, $\phi_2=\tan\beta$, and $\phi_3=-\tan(\alpha+\beta)$. In the final transformation, we have decided on $l=0.8$, $m=-9$. This gives

$$F_1=\frac{\tan\alpha+0.8}{1-0.8\tan\alpha}\ ; \quad F_2=\frac{\tan\beta-9}{1+9\tan\beta}\ ; \quad F_3=\text{etc.}=\frac{1-\tan(\alpha+\beta)}{1+\tan(\alpha+\beta)}\ .$$

The scales on the curve are marked off according to the co-ordinates $x=\frac{F}{F^2+1}$; $y=\frac{F^3}{F^2+1}$.

After making up a table of co-ordinates for the curve from $F=-9$ to $F=+9$, which are the extreme values, it appears advisable to use oblique axes. This may be done by keeping the same abscissas, but using new ordinates $Y=y-15x$, where (x,y) are the old co-ordinates. In this way better intersections are obtained for solutions. In the final form of the chart, the scale for abscissas has been magnified in order to increase the width of the chart (Fig. 8).

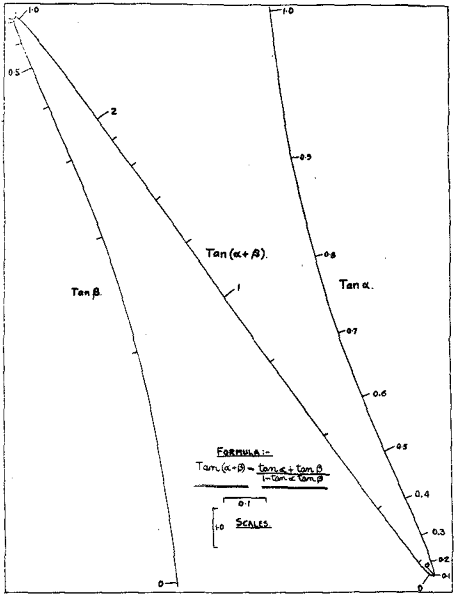


FIG. 8.

Inspection of this chart demonstrates that the cubic is not a practical form. It might be possible to adjust the values of l and m slightly and thus separate the α and $(\alpha+\beta)$ scales, and remove the ends of the three scales from the sharp bends of the curve, but this further adjustment would spread out the tan α scale near the value 1, and the tan β scale near the value 0, and correspondingly close up these scales at the other ends. Of course there are other transformations possible, but practical considerations do not seem to warrant much further study along these lines.

In conclusion, it is interesting to note that the three canonical forms which we have studied may be transformed into one another by the use of either logarithmic or transcendental transformations. Thus $\alpha\beta\gamma=1$ reduces to $\alpha+\beta+\gamma=0$ by taking logarithms, $\alpha\beta\gamma=\alpha+\beta+\gamma$ reduces to $\alpha+\beta+\gamma=0$ by using inverse tangents. The reverse processes are also possible.

A discussion of elementary determinant theory and its application to alignment charts will be found in a previous paper by the author (9, p. 374.)

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THE SORPTION OF VAPORS BY ACTIVE SILICA[1]

By L. M. Pidgeon[2]

Abstract

The sorptive properties of a new active silica have been examined. This sorbent is prepared by the action of acids on the mineral serpentine. The quartz spiral sorption balance has been employed to obtain isotherms for water, benzene and alcohol. Relative rates of sorption have been measured and compared with those for silica gel.

Efficiency measurements have also been made, using the dynamic method, with water vapor as the sorbate. Comparative measurements have been carried out on commercial silica gel, and data are cited from the literature for activated alumina. A comparison of the data indicates that active silica is inferior to silica gel but somewhat better than commercial alumina. Its simple method of production combined with reasonably good sorptive properties should result in a cheap and effective sorbent for technical purposes.

The type of isotherm exhibited by active silica is similar to that associated with the "chalky" gels described by Holmes. Certain theoretical aspects of sorption by "chalky" and "vitreous" gels are discussed.

Introduction

A large number of oxides may be obtained in a porous condition and in this state are known to be good sorbents of vapors and gases. These oxides obtain their porosity from the fact that they are produced by dehydration of the corresponding hydroxide which is generally a colloidal substance. Typical sorbents of this nature are alumina, titania, silica gel, etc. There is, however, another manner in which porosity may be produced but which is not directly connected with such colloidal structure. If the molecules of one component of a complex mixture, such as a naturally occurring silicate, are removed, and the remaining molecules still retain the same position relative to one another, a porous structure is formed which is not a dehydrated gel, yet which exhibits many of its characteristic properties. A well known example of this type of substance is presented in a dehydrated zeolyte such as chabasite, where the water of crystallization has been removed without altering the form of the compound. The resulting structure is enormously porous and capable of a very high degree of sorption.

D. Wolochow has developed in these laboratories a method which results in the production of a similar sorbent from the mineral serpentine. This mineral, which has the empirical formula 3 $MgO.2SiO_2.2H_2O$, when acted upon by dilute mineral acids loses the MgO and water of crystallization almost quantitatively, leaving only the silica and certain naturally occurring impurities. The essential point of the process is that, in the case of serpentine at least, the mineral does not lose its form during the treatment, so that it may be assumed that the silica molecules have the same spatial arrangement after treatment as they had before treatment. The removal of the other

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constituents of the mineral leaves innumerable submicroscopic spaces whose walls present a very large surface.

Preliminary experiments carried out with water at the saturation point indicated a very satisfactory degree of sorptive power, and a more extensive study seemed to be warranted. This paper deals with an examination of the sorptive properties of this substance to which the name "active silica" has been given.

Experimental

The sorption of water, benzene and alcohol vapors has been measured, using a modification of the sorption balance developed by McBain and Bakr (7). This method of measurement presents a number of advantages which need not be enumerated here.

Since the technical interest in this substance lies in its possible applications to commercial processes, a number of experiments have been carried out using the dynamic sorption method, as it is analogous to the conditions holding in the practical application of sorbents in the recovery of volatile solvents and in the drying of air.

I. Static Method

Apparatus. The apparatus is illustrated in Fig. 1, and is in general similar to that described previously (12). All stopcocks were replaced by mercury seals so that organic vapors could be examined. The spirals were mounted as shown in tubes A, B and C which were equipped with mercury-sealed ground glass connections; these were employed successfully in the dry condition. The tubes were immersed as shown in a thermostat, the temperature of which was maintained constant to within 0.02° C.

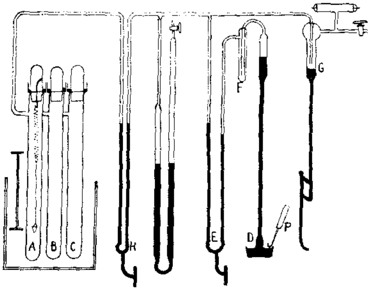

FIG. 1. *Sorption balance.*

The other parts of the apparatus are clearly shown in the diagram. The evacuating system consisted of the usual mercury diffusion pump backed by an oil pump. With this combination it was possible to reduce the pressure in the system to the limit of an ordinary McLeod gauge.

The vapor to be examined was added to the apparatus as a liquid at D. A special pipette, P, was employed in which the liquid was boiled to expel dissolved gases and then introduced under the open end of D; as the apparatus had been previously evacuated the liquid rose to the top of the mercury

column saturating the space *F* with vapor. It was then added to the sorption system as required by opening the cut-off *E*. The liquid could also be frozen and evacuated in the ordinary manner by immersing the tube *F* in a suitable freezing mixture. When a different sorbate was to be employed any liquid adhering to the walls of *D* was removed by gentle heating with a free flame during evacuation.

The quartz spirals were constructed by winding suitable fibres on a fluted carbon rod. A flame was used to soften the fibre at the appropriate point. The average spiral was 1.2 cm. in diameter and consisted of 50 turns. Weights up to 1.5 gm. could be supported, giving a total deflection of approximately 60 mm. Cathetometer readings were accurate to within 0.02 mm.

The samples under examination were placed in buckets constructed of aluminium gauze. The buckets were suspended from the spirals by means of a fine aluminium wire. The elongation caused by these additions was subtracted from the total to obtain the net elongation due to the sample.

Samples. All the samples of active silica which have been examined were prepared by D. Wolochow. They were obtained from serpentine (asbestos tailings) by treatment with hydrochloric or sulphuric acid. The screen sizes of the rock which were used varied from minus 8 to plus 16 mesh. The product is a chalky white substance containing occasional colored impurities. It is relatively soft and may be crushed by hand. The results of an analysis of a well prepared sample are shown in Table I.

TABLE I

RESULTS OF ANALYSIS OF A TYPICAL SAMPLE OF ACTIVE SILICA*

Treatment	Percentages, dry weight basis (ignition)				
	SiO_2	Al_2O_3	F_2O_3	CaO	MgO
H_2SO_4—1 : 3, 6 hr. boiling. Recovery, 44.5%	96.3	1.4	1.0	Trace	1.2

Procedure. The samples were placed in position as shown in the diagram and the whole apparatus evacuated until no further gas was given off by the samples. During this time they lost volatile sorbed gases and vapors and after about eight hours had reached a constant "dry" deflection which was ascertained by means of a cathetometer (both the upper and lower ends of the spiral were read on each occasion to obviate errors consequent on displacement of cathetometer or apparatus).

This "dry" weight is, of course, purely a relative one, but as it is readily reproducible it was chosen in the interests of convenience (see "Discussion of dry weight"). Cut-off *G* was then closed and *E* opened for a time, allowing

* *Analysis by C. W. Davis, Division of Chemistry, National Research Laboratories, Ottawa.*

vapor to enter the apparatus. E was closed, and after a time the pumping system was again operated and the samples were evacuated by opening G. This sequence of events was repeated at least three times and resulted in the "washing" out of inert sorbed gases such as air which might have interfered with the establishment of equilibrium. Vapor was then allowed to enter the apparatus until some suitable pressure was attained, after which the reaction system was closed and allowed to stand until the manometer indicated a constant pressure; readings were then taken with the cathetometer, as previously described.

In this form of the apparatus the pressure in the system falls during the establishment of equilibrium owing to the removal of vapor by the sorbent. These changes are, however, quite small, owing to the large volume of the apparatus compared with the size of samples, and as this fall in pressure is common to most standard sorption methods, the effect has been neglected. For the examination of hysteresis effects, however, it is desirable that the pressure should remain at a reasonably constant value during sorption or desorption at any point; hence a few experiments have been carried out in a previously described apparatus (12) in which these conditions are fulfilled.

Sorption of Water Vapor

1. *The sorption isotherm.* Typical values for the sorption of water vapor by active silica are shown in Table II. These results could be readily duplicated with any given sample although small variations existed among various samples of the same kind.

TABLE II

SORPTION OF WATER BY ACTIVE SILICA

Sample 81; temp., 23° C.		Sample 81; temp., 28° C.		Temp., 20° C. Sample 55		Sample 49
Relative vapor pressure, %	x/m . 100	Relative vapor pressure, %	x/m . 100	$\frac{p}{p_s}$. 100	x/m . 100	x/m . 100
Sorption		*Sorption*		*Sorption*		
8.6	3.0	5.9	2.8	5.3	2.4	2.4
19.7	5.4	16.2	4.9	21.1	4.6	5.4
27.6	7.1	29.3	7.1	41.3	7.3	7.8
41.0	9.3	37.1	8.6	50.0	8.5	9.1
52.8	11.8	47.3	10.5	67.8	11.5	11.7
76.2	17.1	61.1	13.5	82.2	15.3	17.3
95.2	22.8	67.1	15.5	100.0	17.0	19.9
Desorption		*Sorption*		*Desorption*		
100.0	23.8	4.0	2.6	85.1	15.7	18.3
93.4	22.3	23.7	6.2	76.0	14.2	16.1
79.5	18.4	33.1	7.4	61.4	11.5	12.6
61.3	14.0	44.5	10.3	52.8	10.3	10.5
49.0	10.9	55.8	12.3			
31.0	7.2	66.9	14.9			
24.6	5.6	84.0	20.2			
15.1	3.7	100.0	24.6			

One set of values is plotted in Fig. 2, where it produces the familiar sigmoid isotherm which characterizes the sorption of water vapor by a number of well known colloidal substances, such as cellulose, gelatin, etc.

Under the pressure conditions existing in these experiments it was possible to obtain reversible isotherms as seen in Fig. 2. In some experiments, however, there were indications of hysteresis, and, as will be seen later, when the pressure during sorption was not allowed to rise above the final equilibrium value at any point a hysteresis appeared.

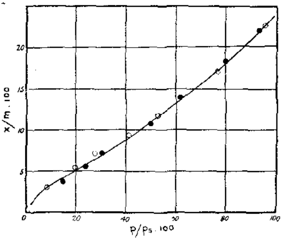

FIG. 2. *Sorption of water by active silica; isotherm at 23° C.*

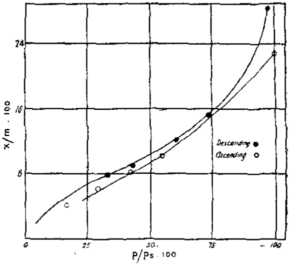

FIG. 3. *Sorption of water by active silica at 20° C.*

Between 20° and 28° C. no difference in sorption is apparent when the results are plotted against relative humidity.

With this type of isotherm, relatively high sorption values should be reached near the saturation pressure owing to the fact that in this region $d(x/m)/dp$ is increasing rapidly. There was evidence of incomplete attainment of equilibrium by sorption near the saturation pressure, as shown by the following experiment. The active silica was wetted with water and the desorption results obtained without preliminary drying. The results of this procedure are listed in Table III and plotted in Fig. 3. The high values which are shown near the saturation pressure are not reached on the subsequent sorption, so that the S-shaped curve is much more obvious when water is being lost than it is when water is being sorbed.

TABLE III

SORPTION OF WATER VAPOR BY ACTIVE SILICA (IN THE WET CONDITION INITIALLY) AT 20° C.

Relative vapor pressure, %	x/m . 100	Relative vapor pressure, %	x/m . 100
Sample containing free water		Sample dried	
Desorption		*Sorption*	
100.0	92.5	16.7	4.1
98.5	41.5	28.7	6.3
97.1	28.4	42.5	8.1
74.1	15.2	54.4	10.1
61.0	12.2	100.0	22.7
43.2	9.2		
33.0	7.8		

The shape of the isotherm is similar to that found in the case of alumina by other workers and similar to that found by Holmes and co-workers in the case of "chalky gels." It differs from the silica gel isotherm which approaches a definite saturation value for sorption at some relative humidity between 60 and 80%.

2. *Hysteresis.* It has been pointed out previously (12) that if hysteresis occurs, its true dimensions should be apparent only if the system as a whole approaches each sorption point strictly in the direction indicated. This result may be obtained only when the pressure in the system during sorption does not rise above that of the new equilibrium point. No simple methods are available to achieve this end, and as the principal aim of this work was to examine the magnitude of sorption by samples of active silica which had been produced by different treatments, the more direct methods of sorption measurement have been followed in most cases. One set of experiments in which the vapor pressure was kept at a constant value during sorption has been performed.

The same methods as those used previously in the case of silica gel were employed. In the first method the vapor pressure in the apparatus was maintained by placing a sulphuric acid solution of known strength in the system. In the second, a body of water in the sorption system was maintained at some constant temperature lower than that of the samples.

In order to obtain comparative results with the same sample under circumstances in which the pressure varied during sorption, a run was carried out in the ordinary manner. That is, the pressure in the system at the moment of addition of vapor was higher than the subsequent equilibrium point, hence sorption took place during a falling vapor pressure. This sequence of events is, of course, reversed during desorption. In order to make the pressure changes more acute a small amount of silica gel was placed in one tube, as the sorption balance, owing to the small size of the samples, shows a lower ratio of sorbent to the volume of system than is common in most other methods.

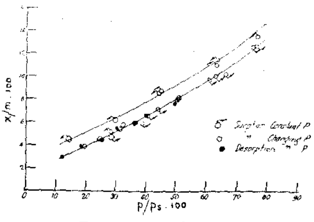

FIG. 4. *Sorption hysteresis.*

Results of this experiment appear in Table IV and Fig. 4, where it will be seen that a hysteresis appears when the vapor pressure is constant, while on the other hand, when fairly large variations appear, the isotherm is reversible. It was unfortunately impossible to continue the hysteresis experiments, hence the results are not sufficiently complete to discuss in detail.

TABLE IV

SORPTION WITH CONSTANT VAPOR PRESSURE

Sorption with constant vapor pressure; temp., 20.38° C.; Sample 32				Sorption with variable vapor pressure; temp., 20.44° C.; Sample 32					
Relative vapor pressure, %	x/m . 100	Relative vapor pressure, %	x/m . 100	Relative vapor pressure, %	x/m . 100	Relative vapor pressure, %	x/m . 100	Relative vapor pressure, %	x/m . 100
Method 1		Method 2							
Sorption		*Sorption*		*Sorption*		*Desorption*		*Sorption*	
29.0	4.9	38.4	5.8	24.6	4.4	49.5	7.6	19.7	3.9
44.1	7.1	40.8	6.5	31.4	5.4	40.8	6.6	32.3	5.9
63.4	10.0	60.8	9.7	36.2	5.8	36.1	5.8	50.2	8.1
76.6	12.6	66.1	10.2	50.2	7.7	30.5	5.6	85.9	14.5
Desorption		75.1	12.5			25.2	4.5		
76.7	13.4	*Desorption*				18.2	3.7		
63.5	10.9	63.4	11.5			12.1	2.9		
44.4	8.5	45.4	8.7						
29.9	6.2	28.6	5.2						
14.2	4.4								

3. *Relative rate of sorption.* The relative rate of sorption in the static apparatus is measured by exposing the dried sorbent to the vapor under consideration at some constant pressure, and measuring the elongation of the spiral at various time intervals until no further change is noticed.

The true rate of sorption cannot be measured in this manner as the measured rate is partly dependent on the speed at which vapor may be evaporated from the liquid surface, and on the time taken for the vapor to penetrate into the lumps of sorbent. The measured rate is therefore a relative value and comparative standards are required to give meaning to the results. Commercial silica gel of about the same particle size has therefore been chosen and examined in the same manner as active silica.

The results of typical experiments of this nature carried out on these substances are shown in Table V.

Fig. 5 shows the rate of sorption as obtained in the static apparatus. It is to be observed that for approximately the first eight minutes the two sorbents take up water at almost the same rate. Active silica rapidly attains a saturation value after the lapse of an hour, while silica gel continues to sorb for

TABLE V

RELATIVE RATE OF SORPTION

Active silica (No. 23)		Silica gel	
x/m . 100	Time, min.	x/m . 100	Time, min.
1.7	2.3	1.3	1.0
1.8	4.3	2.0	2.5
2.8	7.0	2.2	4.0
3.5	14.5	2.6	7.0
4.0	19.5	3.9	11.0
4.8	31.0	5.8	25.0
5.6	55.0	9.5	57.0
5.7	87.0	11.6	117.0
5.7	100.0	12.4	177.0
		13.9	328.0
		14.4	Final equilibrium

NOTE: *Temperature of thermostat, 20° C.; relative vapor pressure, 26.4%.*

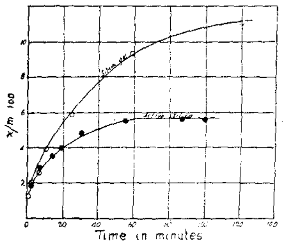

FIG. 5. *Relative rate of sorption at 20° C.; $p/p_s.100 = 26.4$.*

several hours, reaching a much higher final value. This difference in behavior suggests that active silica presents a much more open structure and one in which the innermost parts are readily accessible to the entering vapor. On the other hand, the surface must be greater in the case of silica gel, or at least the agencies which are responsible for the retention of the sorbate are more effective.

The experiments on rate of sorption gave an indication of the time required to establish equilibrium when points on the sorption isotherm were being obtained. When the relative vapor pressure was below 60% two hours was found to be ample. At higher relative pressures longer times were required, while at the saturation pressure 24 hr. was allowed to elapse.

4. *Effect of acid treatment of serpentine on sorption.* Since the sorptive properties of active silica are due to the removal of magnesium oxide and water from the parent mineral in such a way that the silica remains in the same form, the completeness of removal of the soluble constituents should exert a definite effect on sorption. In order to examine the optimum conditions of treatment, a number of complete isotherms have been obtained using silica that had been produced by different acid treatments. Points taken from these curves are listed in Table VI, together with details of the treatment accorded in each case.

TABLE VI

EFFECT OF ACID TREATMENT ON SORPTION

Sample No.	Acid	Conc.	Time of boiling, hr.	% Recovery	p/p_s 20%	50%	70%	95%
					x/m	x/m	x/m	x/m
23	H_2SO_4	1 : 3	3	47.8	5.8	10.5	13.2	—
32	H_2SO_4	1 : 3	6	44.5	6.1	11.0	15.4	22.5
49	H_2SO_4	1 : 3	16	42.7	5.6	10.0	14.2	19.2
39	H_2SO_4	1 : 3.5	3	46.3	5.4	10.0	13.8	19.0
55	H_2SO_4	1 : 2.75	3	45.6	4.5	9.0	12.6	18.2
66	HCl	20%	2	47.8	5.9	10.5	15.8	22.7
72	HCl	20%	4	46.2	5.0	10.2	14.5	20.8
86	HNO_3	35%	2	51.7	4.5	8.8	12.0	16.8
87	HNO_3	40%	3*	57.3	4.0	8.0	10.7	14.0

*60-90°C.

The empirical formula for serpentine is $3\ MgO.2\ SiO_2.2H_2O$. On the basis of molecular weight, and neglecting impurities such as iron, etc., complete removal of the magnesium oxide and the water of crystallization would

result in a recovery of 43.3% of the original weight of the mineral. Hence it will be seen that the results of the sulphuric acid treatments described in Table VI have approached this theoretical limit and in one case have actually passed it. The sorptive power seems to follow the degree of removal of soluble constituents. One exception to this appears in the table in the case of Sample 49, which was given the most vigorous treatment of all. There appears to be no appreciable difference between the action of hydrochloric acid and that of sulphuric acid, but nitric acid is definitely less effective, probably because the mineral is less soluble in this acid.

5. *Discussion of "dry" weight.* Active silica, like silica gel and many other sorbents, retains a certain amount of water in the so-called "dry" or "activated" condition. This water is associated with the solid in such a manner that it cannot be removed without resorting to drastic methods of heating which, if carried out, may permanently impair the sorbent. These conditions are not generally imposed and the "dry" point or "activated" condition, on which all sorption values are based, is an arbitrary value the magnitude of which is dependent on the conditions that are chosen.

In the experiments which have been described the samples as received had been heated to 120° C. and stored in airtight bottles. They were placed in the sorption balance without further treatment and evacuated until a constant weight was reached. This point was found to be reproducible in any given experiment although, as seen in Table VII, as much as 9% of water remains if the weight at 1000° C. is taken as zero. Table VII also contains values for silica gel, which shows a similar behavior. The sorptive power is reduced by such treatment but, owing to its rigid structure, active silica is less affected by high temperature than is silica gel. Since any reproducible point will obviously serve as a "zero" it is unnecessary to resort to this drastic treatment in every case.

TABLE VII*

"DRY" WEIGHT OF ACTIVE SILICA AND SILICA GEL

Temp., °C	% Water			
	Active silica			Silica gel
	1	2	3	
120	9.2	8.1	7.8	7.4
360	6.9	7.2	5.8	4.7
600	3.3	2.1	2.9	2.0
700	1.2	0.4	0.7	0.6
850	0.2	0.4	0.3	0.1
1000	0.0	0.0	0.0	0.0

**Supplied by D. Wolochow.*
NOTE: *Weight at 1000° C. taken as zero.*

Sorption of Organic Vapors

The sorption of benzene and ethyl alcohol vapors has been examined in a manner similar to that described for water.

Tables VIII and IX contain typical results of these experiments. The magnitude of sorption is noticeably less than in the case of water, though the isotherms present a number of common features (see Fig. 6).

TABLE VIII

SORPTION OF BENZENE VAPOR BY ACTIVE SILICA AT 23° C.

Relative vapor pressure, %	x/m . 100	x/m . 100
Sorption		
11.9	5.5	5.9
25.2	8.0	8.9
32.8	8.4	9.4
65.0	12.9	13.5
74.0	13.9	15.1
100.0	16.3	16.7
Desorption		
83.5	15.5	15.5
19.9	7.2	7.5
6.1	4.4	4.4

TABLE IX

SORPTION OF ETHYL ALCOHOL VAPOR BY ACTIVE SILICA AT 23° C.

Relative vapor pressure, %	x/m . 100	x/m . 100
Sorption		
6.5	3.2	3.7
33.8	6.1	5.7
54.9	8.3	8.2
79.3	12.2	11.8
100.0	15.5	15.8
Desorption		
83.3	13.3	13.3
61.5	10.0	10.1
48.8	7.8	7.8
30.7	5.9	5.8
16.0	4.6	4.3

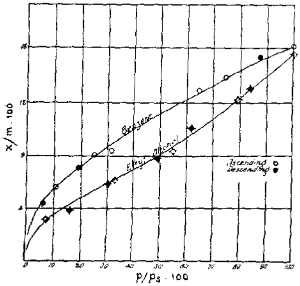

FIG. 6. *Sorption of benzene and alcohol by active silica at 23° C.*

No hysteresis was observed during the sorption of benzene or ethyl alcohol by active silica, the "ascending" or "descending" points falling on the same curve. The shape of the alcohol isotherm is distinctly reminiscent of the water curve showing the same sigmoid shape, resulting in relatively higher values for sorption near the saturation point. The curve for benzene, on the other hand, is rather like the type of curve obtained with silica gel. These differences are emphasized when the isotherms are plotted according to various sorption equations (see Discussion).

In Tables VIII and IX the values have been listed for the two samples which were examined concurrently in this particular experiment. Complete values have been given to bring out a very interesting fact which was frequently noticeable during the sorption of these organic vapors. In Table IX it will be seen that much closer agreement exists between the two samples during desorption than during sorption. The values in the former case are almost invariably identical while, during sorption, variations as large as 1.2% are evident. That these variations cannot be due to experimental error is shown by the accuracy of the desorption points. Hence they must be connected with the sorption process itself. This fact may have a bearing on the theory of sorption. It seems that in order to explain the process of sorption some "condensation" of the sorbed phase must be postulated, employing this term in its widest sense. It appears that as the molecules are removed

from this condensed state, more regular and uniform conditions follow than when they assume it. That is, values of sorption obtained on a "descending" curve are likely to be more uniform and repeatable than those obtained on an ascending curve.

II. Dynamic Method

Though the sorption isotherm is the ultimate indication of the sorptive power of any substance, it fails to give certain information which is desirable for the practical utilization of the sorbent. This is because it considers only final equilibrium values and in practice these are seldom attained, as a sorbent which is completely saturated at any relative vapor pressure has, in effect, ceased to be a sorbent. Hence for technical purposes, sorbents are frequently compared with regard to their efficiency, that is, their power to remove a sorbate from a stream of air or other indifferent gas. When the removal is complete and the exit gases show no trace of the sorbate the efficiency is 100%. When this condition no longer holds and vapor appears in the outlet gas, the sorbent has reached the "break point"; it will, of course, continue to take up vapor until the saturation value corresponding to the particular partial pressure and temperature is reached, but in practice it is not desirable to exceed the "break point," as an appreciable loss of vapor will occur.

Efficiency may be measured by ascertaining the time during which the removal of vapor by a given amount of sorbent under definite conditions is complete. The dynamic sorption apparatus illustrated in Fig. 7 was employed. The saturator consisted of a triple bubbler fitted with fluted filter paper to increase the liquid surface. The vapor pressure was adjusted by placing the saturator in a separate thermostat. Suitable spray traps were interposed. The vapor-air mixture passed from these through a copper coil immersed in the primary thermostat to ensure that the mixture entered the sorption tube at the same temperature as the sorbent.

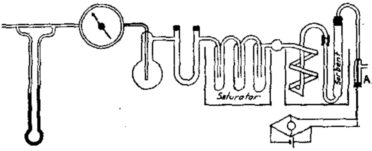

FIG. 7. *Dynamic sorption apparatus.*

The experiments were carried out using water, as the sorbate, the presence of moisture in the air leaving the sorbent being detected by a device described by Anderson (1) and shown at *A*, Fig. 7.

A small globule of calcium chloride is fused between two copper wires placed 1 mm. apart. The wires are placed as the unknown in a Wheatstone bridge circuit. The device is arranged so that the air to be tested must pass over the calcium chloride. With dry air the resistance of the circuit was about 100,000 ohms, while a trace of moisture reduced this value to about 500 ohms. An a-c. bridge circuit gave the most satisfactory results. It was unnecessary to fuse the globule between successive determinations as it

could be readily dried by passing dry air through the tube. When dry air passing through the apparatus was replaced by air at a relative humidity of 25%, the detector responded actively to the change in approximately five seconds.

The sorption tube containing some 4 gm. of the sample to be examined was placed in an oven at 155° C., and a gentle stream of dry air was passed through the tube for two hours. After the tube was weighed it was placed in the sorption train and moist air was drawn through at a known partial pressure until the presence of water vapor in the outgoing gas indicated that the sorbent had ceased to operate at 100% efficiency. The time, amount of gas passed, and the weight of the sample were noted. A typical set of results are to be found in Table X.

TABLE X
EFFICIENCY OF ACTIVE SILICA AT 23°C.

Rel. hum., %	Vol. of air, litres	Time, min.	m, gm.	x, gm.	$x/m \cdot 100$
96.0	31.91	38	4.226	0.5110	12.1
96.0	34.4	42	4.2170	0.4975	12.1
96.0	29.8	37	4.2230	0.4930	11.7
26.3	100.0	120	4.2230	0.3410	8.1
26.3	98.3	112	4.2210	0.3240	7.8

The effectiveness of this sorbent may be seen from the fact that its volume was approximately 5 cc. which, with an average gas velocity of 830 cc. per min., resulted in a time of contact between gas and sorbent of approximately 0.3 sec., yet the removal of water vapor was complete for the times shown in Table X. After the appearance of moisture at the outlet the silica continues to take up water until the composition of the outlet gas is the same as that of the entering gas. This point will be the equilibrium point for the particular vapor pressure and temperature and will correspond to that appearing in the isotherm given above, subject, of course, to correction for the different manner in which the "dry point" was obtained.

Comparison of active silica with other common sorbents. Table XI shows a comparison of the saturation values for active silica with those of some other more well known substances commonly used as sorbents. The values for silica gel were obtained in this laboratory with the sorption balance, and refer to the commercial product. Values for activated alumina have been taken from the literature on the subject. Other values for alumina are available, some showing higher values than those listed in Table XI. The saturation value in the case of water is corroborated in a pamphlet published by the makers of the commercial product.*

TABLE XI
SORPTION SATURATION VALUES FOR VARIOUS SUBSTANCES—SATURATION VALUES AT 25° C.; % BY WEIGHT

Sorbate	Alumina (10)	Active silica	Silica gel
Water	23.0	25.0	33.0
Benzene	15.7	17.2	25.0
Ethyl alcohol	12.9	15.2	19.1

**The Al minium Company of America. Properties of activated alumina. 1930.*

The same order as that appearing in Table XI is shown when the relative afficiencies are considered. Silica gel has been tested as described above end it was found that when saturated air passed through the gel at a rate of 100 cc. per min. per gm. of gel, the removal of water vapor was complete for 108 min., during which the gel took up 21% of its weight of water vapor. This value checks well with that given by Miller (9), who employed a different method of moisture detection.

Active silica at 100 cc. per min. per gm of gel remained 100% efficient for 76 min. and at the end of this time had taken up 12.1% of its dry weight. Similar values for alumina were not measured but the makers of the commercial product* prescribe a rate of 10 cu. ft. per hr. per lb. of alumina, under which circumstances the gel will sorb water at 100% efficiency until water is taken up to the extent of 8 to 10% of the dry weight of the sorbent. This rate is much lower than that stated above, but the amount of sorption, when water vapor first appears in the outlet gas, is in accord with the values as obtained from static experiments and indicates that commercial alumina is somewhat inferior to active silica as a water absorbent.

In general it seems that this new sorbent should prove useful in technical processes, as its sorptive power compares favorably with other sorbents in common use. It may be readily reactivated and appears to maintain its desirable properties even when heated to relatively high temperatures. As it is produced from a natural product by a simple chemical reaction, its manufacturing cost should be less than that of most of the sorbents which have been exploited up to the present.

Discussion

The method of production of active silica consists in removing the acid-soluble components of a hydrated magnesium silicate in such a manner that the silica remains in the same crystal arrangement as before, or at least the mineral retains its original shape.

It is of interest to note that while this sorbent is obtained from a natural product, a somewhat analogous compound has been prepared in the laboratory. Holmes (5) describes a sorbent made by precipitating silicate of soda with ferric chloride under such conditions that ferric hydroxide is deposited in the pores of the silicic acid gel. "The novel feature of this process is the removal of ferric hydroxide by acid *after* the gel has been dried to a rigid non-collapsing structure. The traditional silica gel obtained capillarity by loss of water molecules."

It is at once apparent that this process is analogous to the production of active silica in which the "rigid non-collapsing structure" is the mineral serpentine. The product produced by Holmes has been called "chalky gel" to differentiate it from the hard glassy silica gel. Though their methods of production are somewhat different, both the physical properties and type of sorption of the "chalky" gels are very similar to those of active silica. They

* *The Aluminium Company of America. Properties of activated alumina. 1930.*

are both opaque, chalk-like substances, rather easily broken and quite different from silica gel which shows the hard, horn-like appearance which characterizes a dehydrated non-swelling gel.

Holmes (6, 3) showed that very high values of sorption could be attained by his gel at the saturation point of water and other vapors, values which were greatly in excess of those found in the case of silica gel, but owing to the shape of the isotherm, they were maintained only in the vicinity of the saturation point, and at lower relative vapor pressures vitreous silica gel showed higher sorption. The isotherm for vitreous silica gel tends to approach a definite saturation value after which no more sorption takes place. The curves obtained with Holmes' gel, on the other hand, show the same shape as those presented for active silica, so that relatively high values for sorption may be reached as the saturation pressure of the vapor in question is approached. (The actual values for "chalky" gels are much higher than for active silica). Owing to the shape of the curve in this region $d(x/m)/dp$ is changing so rapidly with pressure that the sorption value at the saturation point is indefinite.

Reference to the curves for active silica and water will show that the isotherms possess the characteristic shape of those of the "chalky" gel type. It appears therefore that if porosity is produced by the removal of a metal constituent from a silica mixture, the resulting sorption isotherm is such that very high values are attained as the saturation pressure is approached. In the case of the "vitreous" silicic acid gel, porosity is produced by the removal of water molecules, and the isotherm tends to show a definite saturation at some pressure below the saturation pressure of the liquid.

These two types of isotherms have been referred to previously, and it is of interest to note that in this case they appear with the same substance, depending on the manner in which it was prepared. The shape of the isotherm may also depend on the vapor, as it has been shown that benzene gives the C-shaped curve, while water gives the sigmoid curve with active silica. Munro and Johnson (11) have found similar results in the case of sorption by alumina. They point out that "when the vapor pressure is two-thirds of the saturation pressure, the gel has taken up only one-fifth of the saturation amount of water, whereas three-fourths of the total quantity of benzene is held at this relative pressure."

It is clear that no single sorption equation will fit these isotherms throughout their whole length. The ordinary empirical sorption formula, $x/m = ap^{\frac{1}{n}}$ is applicable in certain cases, if the lower portion only of the isotherm is considered. In isotherms of the type shown by the benzene-silica gel system, it fails completely, the logarithmic plot being a flat curve concave to the pressure axis. The flat curve changes to a horizontal line at higher relative vapor pressures. The curves obtained with active silica, on the other hand, show some agreement with the formula at relative vapor pressures less than 60%, while the silica gel-water system shows agreement up to 50% relative vapor pressure. These curves are illustrated in Fig. 8.

McBain (8) has shown that in the case of charcoal, good agreement with the observed results is obtained by application of the Langmuir formulation, $x/m = \frac{abp}{1 + ap}$. The isotherms for silica gel and benzene are similar to the charcoal type in that most of the sorption takes place before half the saturation pressure is reached. The various isotherms for silica gel have been plotted according to this formula in Fig. 9, the values for silica gel having been taken from a previous paper (12). It will be seen that agreement exists in the case of the alcohol and benzene systems, though water shows no correlation. Active silica shows some agreement in the case of benzene, but of course the sigmoid isotherm appearing in the water and alcohol systems cannot be represented by the Langmuir equation.

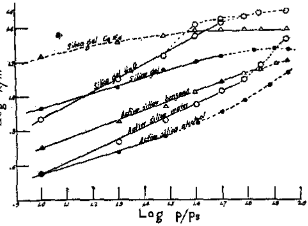

FIG. 8. *Logarithmic plot of isotherms.*

McBain has amply demonstrated in the case of charcoal that the concept of capillary condensation may not be applied to systems where most of the sorption takes place at low relative vapor pressures. It seems that his argument may be logically extended to the silica gel-benzene system, so that sorption in this case may be considered as a direct surface phenomenon which ceases when the available surface has become saturated. The chalky gels, however, show the characteristic increase in sorption near the saturation point. This suggests that condensation of liquid is taking place in this region at least.

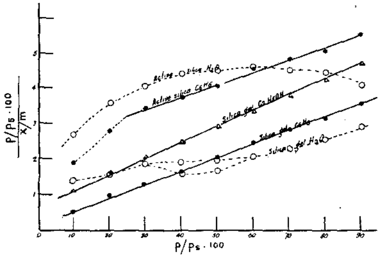

FIG. 9. *Isotherms plotted according to Langmuir equation.*

In general the amount of sorption which takes place at any temperature is governed by the natures of the gas and the solid, the partial pressure of the sorbate, and the characteristic structure of the solid. The systems under discussion present a case in which the fundamental natures of the solids are the same, yet the type of sorption is quite different at higher partial pressures. Condensation of liquid appears to be possible with the "chalky" gels but not with "vitreous" gels, as in the latter case sorption rises to a maximum before the saturation pressure is reached. If both solids are assumed to be of the

same nature, the differences in behavior must be due to a different gel structure. It is generally assumed that the sigmoid isotherm is produced by a sorbent of larger pore diameter.*

In this connection it is interesting to note that Freundlich (4) has suggested that differences in the shape of isotherms near the saturation pressure may be due to the wetting power of the condensed phase for the solid. When the condensed phase readily wets the solid, liquid may appear and the amount of sorption rises very sharply, reaching high values at the saturation point. On the other hand, if the liquid does not wet the sorbent, or does so with the formation of an angle of contact, then the vapor behaves as a gas above its critical temperature, and no increase in sorption takes place and the isotherm does not rise near the saturation point. It should follow that an isotherm of the "wetting" type would change to the "non-wetting" type above the critical temperature. Very little work of this nature has been carried out owing to the experimental difficulties which are involved and, in such experiments as have been carried out, sorbents showing the "non-wetting" type at ordinary pressures have been employed.

The actual nature of the pore walls may bring about this difference, as shown by Bonnell (2) in the case of silica gel. He found that the undialyzed gel produced the isotherm giving high sorption values at the saturation point, though the dialyzed gel gave the ordinary isotherm showing hysteresis. These results were obtained even when the gels were dried; hence the acid remaining on the pore walls brought about the significant change in the type of sorption.

Acknowledgment

The author wishes to acknowledge his indebtedness to Mr. D. Wolochow, of the Division of Chemistry, National Research Laboratories, who developed the method of preparation of the active silica and who prepared all the samples; and to Dr. G. S. Whitby, Director of the Division, at whose suggestion this investigation was carried out.

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THE MOLECULAR DIAMETER OF DEUTERIUM AS DETERMINED BY VISCOSITY MEASUREMENTS[1]

By A. B. Van Cleave[2] and O. Maass[3]

Abstract

The viscosities of deuterium over the range 23 to −183°C. have been measured. The viscosities of the two-component system deuterium-hydrogen have been measured over the whole concentration range at 22°C. The results show that the deuterium molecule has the same diameter as the hydrogen molecule. The interest attached to the viscosity results for the two-component system are pointed out. The difference between the molecular volume of liquid deuterium oxide and that of liquid water is attributed by the authors to a difference in equilibrium between associated and non-associated molecules in the respective systems.

Experimental

The apparatus used in the viscosity determinations was essentially the oscillating disc type described in detail by Sutherland and Maass (2). A few minor improvements have been made. These will be described in a subsequent paper. This apparatus is ideally suited to the measurement of viscosity coefficients where only small amounts of gas are available. Besides, it has the advantage of affording an exceedingly accurate method of measuring gaseous viscosities at all temperatures below room temperature. It is estimated that the values given below are correct to better than 0.20%, and have a relative accuracy of 0.10%.

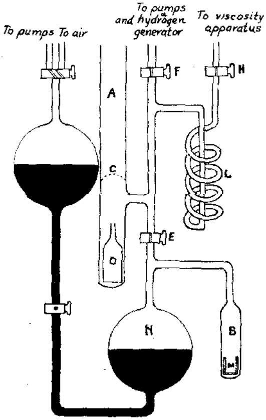

Fig. 1. *Diagram of apparatus used in preparation and purification of deuterium.*

Preparation and Purification of Deuterium

The deuterium was prepared from one gram of deuterium oxide (sp. gr. 1.1058), which was purchased from the Ohio Chemical and Manufacturing Company. The deuterium was liberated by allowing the oxide to react with pure metallic sodium. Fig. 1 is a diagram of the apparatus used in the preparation and purification of the deuterium.

The apparatus was first evacuated and filled with dry air. The tip was then cracked off the deuterium oxide container, *D*, which was quickly lowered into the tube *A*. *A* was then immediately sealed off at *C*. By making the tube *A* long, the sealing off could be accomplished without causing condensation of any

[1] *Manuscript received December 29, 1934.*
Contribution from the Department of Chemistry, McGill University, Montreal, Quebec, Canada.
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water, from the flame, on the inside of the tube. The deuterium oxide was then frozen in liquid air and the whole system evacuated and flushed with pure dry hydrogen. The frozen deuterium oxide was allowed to melt in the presence of the hydrogen to expel dissolved air. Tube *A* was again immersed in liquid air and the system thoroughly evacuated. Tube *B* containing the aluminium cup *M*, nearly filled with metallic sodium, was then surrounded by liquid air while *A* was allowed to warm up to room temperature. When all the deuterium oxide had sublimed into *B* the system was again evacuated and the stopcock *E* closed. The liquid air was then removed from *B*, allowing the reaction to take place.

The deuterium was collected over very pure mercury in the 500 cc. bulb *N*. When *N* was nearly filled with gas the reaction was stopped by replacing the liquid air around *B*. Stopcock *E* was then opened, *H* and *F* being closed. The coil *L* was surrounded by a Dewar flask containing liquid air. Stopcock *H* was then partly opened and the deuterium slowly forced into the evacuated viscosity apparatus. Previous to evacuation the viscosity apparatus had been flushed with pure, dry hydrogen. The reaction in *B* was then allowed to proceed, being aided by heating to 200°C. In this manner enough deuterium was obtained to fill the viscosity apparatus to a pressure slightly greater than atmospheric at room temperature. The gas pressure in the viscosity apparatus could be read on an attached absolute manometer.

The method of making the deuterium-hydrogen mixtures was as follows. Part of the original deuterium was removed from the viscosity apparatus and the pressure noted. Very pure hydrogen was then admitted and the pressure again read, the temperature being maintained constant during this operation. The molecular percentages of the constituents were calculated directly from their partial pressures. All mixtures were allowed to stand for 24 hr. before making any viscosity determinations, thus insuring perfect mixing of the two gases.

Results

Before making any measurements on deuterium, check determinations were made on pure hydrogen prepared by two different methods:— (a) hydrogen from a Kipp generator, purified as indicated by Sutherland and Maass (2); and (b) hydrogen prepared by allowing pure distilled water to react with metallic sodium, as outlined above. In all cases the values for the viscosity coefficients agreed with those given by Sutherland and Maass (2) within 0.16%. This affords an excellent check on the purity of hydrogen prepared by the sodium method.

Since the specific gravity of the original deuterium oxide was 1.1058 it contained 98.0% of D_2O by weight. Hence, on a basis of molecular percentages the deuterium formed 96.08% of the mixture, the remainder being ordinary hydrogen.

Table I is a summary of the results obtained with this mixture. Each value is the mean of three or four independent runs. These results are shown graphically in Fig. 2.

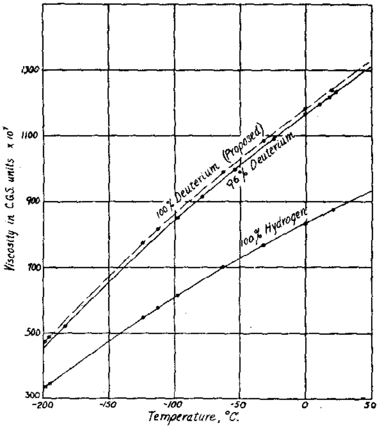

FIG. 2. *Variation of viscosity with the temperature.*

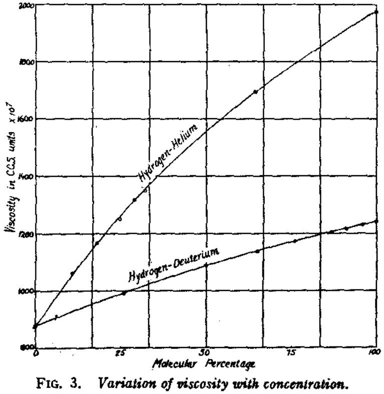

FIG. 3. *Variation of viscosity with concentration.*

TABLE I

Variation of viscosity with temperature (98.0% by weight deuterium, 2.0% by weight hydrogen

Temp., °C.	$\eta \times 10^7$	Temp., °C.	$\eta \times 10^7$
23.61	1234.9	−24.23	1092.1
18.11	1218.9	−53.90	998.7
10.37	1196.3	−79.13	914.7
−0.19	1165.3	−183.00	520.0

Table II shows the result of diluting the deuterium with hydrogen. For comparison all the experimental values have been reduced to their values at 22.0°C. (fourth and eighth columns). As before, each value is the mean of at least three independent runs. The value for 100% deuterium was obtained by extrapolation. These results are graphically represented in Fig. 3.

TABLE II

Viscosities of deuterium-hydrogen mixtures

Deuterium, molecular per cent	Temp., °C.	$\eta \times 10^7$	$\eta_{22°} \times 10^7$ (calcd.)	Deuterium, molecular per cent	Temp., °C.	$\eta \times 10^7$	$\eta_{22°} \times 10^7$ (calcd.)
1.0000	22.00	—	1242.5	.6513	22.06	1138.3	1138.2
.9608	22.47	1232.4	1231.0	.4998	21.96	1088.2	1088.3
.9111	22.00	1217.0	1217.0	.2564	22.02	991.3	991.3
.8677	22.07	1205.1	1205.0	.0000	22.00	878.8	878.8
.7634	21.73	1173.6	1174.3				

Discussion

The well known expression

$$\eta = 1/3mnxl$$

can be reduced to $\eta = kM/\sigma^2$ for purposes of this discussion. η is the coefficient of viscosity of a gas, m the mass of one molecule, n the number of molecules in 1 cc. of gas, x the average velocity of the molecules, l the mean free path, M the molecular weight, σ the molecular cross section, and k a constant for a given temperature. Hence, we have

$$\frac{\eta_D}{\eta_H} = \sqrt{\frac{M_D}{M_H}} \cdot \frac{\sigma_H^2}{\sigma_D^2} ,$$

where the D subscripts refer to deuterium and the H subscripts to hydrogen. If $\sigma_D = \sigma_H$ then

$$\eta_D = \sqrt{\frac{M_D}{M_H}} \cdot \eta_H = \sqrt{2} \cdot \eta_H .$$

The extrapolation of the deuterium-hydrogen curve in Fig. 3 shows that the viscosity of deuterium at 22°C. is $1.414 \pm .002$ times the viscosity of hydrogen at that temperature. Thus, within the writers' experimental error the radii of deuterium and hydrogen molecules at 22°C. are the same.

The ratio $\eta_D : \eta_H$ was found to be constant over a large temperature range. and hence the molecular volumes of deuterium and hydrogen are the same.

In this connection it is of interest that the molecular volumes of liquefied D_2O and H_2O are not the same.

Lewis and MacDonald (1) give the specific gravity of pure D_2O at 25°C. as 1.1056, as against 1.111 calculated by assuming that D_2O has the same molecular volume as ordinary water. This means that the apparent molecular volume of liquid D_2O is greater than that of liquid H_2O.

If this were due to the difference between the volume of the deuterium atom and that of the hydrogen atom then, since the oxygen contributes a large part of the volume in the oxides, the difference would have to be greater than 1%. The viscosity experiments show that as far as the deuterium and hydrogen molecules are concerned a difference of such magnitude does not exist. To account for the difference between the molecular volume of liquid D_2O and that of liquid H_2O, the authors advance the hypothesis that the D_2O and H_2O molecules have the same volume but that in the equilibria

$$3\ D_2O \rightleftharpoons (D_2O)_3 \quad (1)$$

$$3\ H_2O \rightleftharpoons (H_2O)_3 \quad (2)$$

the first is displaced more in the direction of association than the second. In that case the difference in molecular volume ought to become less with rise in temperature, as this favors the formation of the unassociated molecules. Lewis and MacDonald found this to be the case.

Sutherland's equation fails to represent the variation of viscosity with temperature for most gases at low temperatures. The results of Sutherland and Maass (2) for hydrogen and the authors' results for deuterium do not conform to this equation. The relation proposed by Jean's, $\eta/\eta_0 = \left(\frac{T}{T_0}\right)^n$, gives the best representation of the viscosity variation of hydrogen and deuterium over the temperature range of 25°C. to −80°C. With $n = 0.699$ the calculated values for the viscosity of 98.0 weight per cent deuterium check exceedingly well with the experimental values over this temperature range. However, at −183°C. the deviation of calculated from experimental value is 3.13%. Similar results are obtained when this equation is applied to pure hydrogen.

The whole question of temperature variation of viscosity at low temperatures is an interesting one, and the data accumulated in this laboratory will be published shortly.

From the curves in Fig. 2 it is found that over the temperature range 25° to −80°C. the 96 molecular per cent deuterium curve can be obtained from the 100% hydrogen curve by multiplying values on the latter by $1.395 \pm .003$. Below −80°C. this relation begins to fail until at −183°C. the 96 molecular per cent deuterium has a viscosity of only $1.368 \pm .003$ times that of hydrogen.

The proposed viscosity curve (Fig. 2) for 100% deuterium was constructed by extrapolation. From 25°C. to −80°C. this curve should be very nearly

the correct one. Deviations from the true values will be greater at the lower temperatures. Table III gives values taken from this curve.

The viscosities of gas mixtures are of considerable interest from the point of view of the kinetic theory. Deuterium-hydrogen and deuterium-helium mixtures are of particular importance. Now that it has been shown that the cross section of the deuterium molecule is the same as that of the hydrogen molecule, the approximations of the mean diameters necessary in some theoretical deductions are unnecessary. The first system therefore is one in which molecules of equal diameter but different weight influence the viscosities. In the second system the molecules are of equal weight but have different molecular diameters. The data for the first system are shown graphically in Fig. 3. In this figure the data for the hydrogen-helium system, as determined by Trautz and co-workers (3, 4), are given for purposes of comparison. The authors intend to obtain the data for the deuterium-helium system, and when this is done the relations deduced for mixtures by Trautz, Chapman and others will be critically examined. Preliminary calculations on the system deuterium-hydrogen seem to show that further amplification of the theory is necessary.

TABLE III

VISCOSITIES OF DEUTERIUM AT DIFFERENT TEMPERATURES

Temp., °C.	$\eta \times 10^7$	Temp., °C.	$\eta \times 10^7$
20	1240	−100	861
0	1181	−120	787
−20	1120	−140	710
−40	1060	−160	632
−60	997	−180	550
−80	931	−200	468

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VISCOSITY OF GLUTEN DISPERSED IN ALKALI, ACID AND NEUTRAL SOLVENTS[1]

By R. C. Rose[2] and W. H. Cook[3]

Abstract

The viscosity of gluten dispersed in urea and sodium salicylate was higher, and showed a greater increase with increasing protein concentration, than that of dispersions of the same age and concentration, in sodium hydroxide and acetic acid solutions. Calculations, based on these measurements, indicated that the effective particle size is larger in the former pair of solvents. In urea solutions the viscosity of dilute gluten dispersions was independent of the hydrogen ion concentration between pH 6.1 and 9.2, and within this range the system was stable. Beyond this pH range the viscosity at first increased, but the system was unstable as shown by a subsequent rapid decrease in viscosity with time.

Dilute dispersions in sodium hydroxide, urea and sodium salicylate solutions decreased in viscosity at first, whereas the viscosity of dispersions in acetic acid decreased continuously. Some evidence was obtained of coagulation in concentrated dispersions in the neutral solvents at 0° C. and 25° C.

The character of the precipitate obtained by salting out dispersions in each of the four solvents after storage at 25° C. indicated that the neutral solvents alter the gluten less than alkali or acid. This conclusion is supported by the fact that glutens obtained from flours of different protein quality had essentially the same viscosity when dispersed in alkali or acid, but in the neutral solvents exhibited markedly different viscosities which were partially correlated with the quality of the gluten.

1. Introduction

Viscosity has been used in this study as a measure both of the state of, and of the change in, dispersions of gluten in various solvents. It has been shown by Cook and Alsberg (7) and Cook and Rose (8) that wet gluten, obtained from wheat flour, can be dispersed completely in solutions of urea and sodium salicylate. The dispersing action of these solutions on coagulated proteins had already been demonstrated by other investigators (1, 11, 25) some of whom also showed that they denature, or otherwise alter, albumin and haemoglobin. In the present investigation of the viscosity of gluten dispersed in these, and in the classical solvents, dilute alkali and acid, an effort was made to determine the relative extent to which gluten was altered by dispersion in these four reagents.

A decrease in solubility is generally the first indication that a protein has been altered by a given treatment. The derivatives produced, namely, proteans, metaproteins and coagulated proteins, are defined, for the most part, by the nature of the treatment which produces them, rather than by any characteristic property of the resulting substance. It is probable that any difference in the solubility of these products represents a difference of degree rather than of kind.

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The coagulation of proteins has been studied more extensively than any of the other changes. Most of these investigations have been made on albumin or haemoglobin and, since these proteins can be crystallized, it appears to be reasonably certain that they are not altered in preparation. On the other hand, with other classes of proteins there is a possibility that the method of extraction and purification may alter the material to some extent before it can be studied systematically (15). Albumins can be coagulated in many different ways and it is not known whether the coagulation produced by these different methods is the same. The classical theory of coagulation is that it is the result of two distinct changes, denaturation and flocculation. The exact nature of the former is obscure but is believed to be chemical, while flocculation is regarded as a purely colloidal process which takes place under suitable conditions of hydrogen ion concentration and in the presence of certain electrolytes. Wu (26) and Wu and Chen (27) distinguish between the material produced by denaturation in acids and alkalis which flocculates at the isoelectric point, and the coagulum produced by the action of alcohol, heat, shaking, etc., in the isoelectric region. They (28) pointed out later that denaturation of certain proteins, including egg albumin, is accompanied by the liberation of non-protein substances, whereas coagulation is not. Wu regards denaturation as degradation, and coagulation as condensation. On the other hand, Sørensen and Sørensen (23) have shown that no nitrogen compounds of any kind are liberated from egg albumin during denaturation. In view of the conflicting evidence it seems probable that degradation is the result of secondary changes rather than of denaturation proper.

From the physical standpoint, the viscosity measurements of Anson and Mirsky (3) and Loughlin and Lewis (16) indicate that the process of denaturation results in some change in the state of aggregation even when flocculation is absent. Other work of Anson and Mirsky (2, 19) also indicates that denaturation of albumin and haemoglobin is not irreversible as it was once thought to be. The method which they employed for reversing denaturation suggests a physical rather than a chemical change. Hewitt (10), however, has questioned the method of denaturation employed by Anson and Mirsky and consequently their method of effecting reversal may be of little value in interpreting the nature of the changes involved. Further support for the view that denaturation is a colloidal phenomenon is given by the work of Bancroft and Rutzler (4).

The action of acid and alkali on proteins was discussed briefly in an earlier paper (7) and it is sufficient to mention here that the work of Sjögren and Svedberg (22) and Speakman and Hirst (24) has shown that the stability of such soluble proteins as albumins and of such insoluble proteins as keratin is restricted to a limited range of hydrogen ion concentration. It is also well known that gluten, like many other proteins, exhibits a maximum swelling and viscosity at hydrogen ion concentrations remote from the isoelectric point. The work of Sharp and Gortner (21), however, suggests that the high viscosity of gluten at pH 3.0 and pH 11.0 is an unstable transitory

condition, but it is not known whether in its final condition at these hydrogen ion concentrations the protein is denatured or otherwise degraded. The evidence presented by Bungenberg de Jong (6) also suggests that the properties of gluten are changed at hydrogen ion concentrations remote from its isoelectric range.

It is questionable whether the methods used in, and the results of, previous investigations of the denaturation of other proteins can serve to elucidate the changes which occur in the denaturation of gluten. It seems probable that the term 'denaturation' denotes different changes in different proteins, and consequently the changes which occur in gluten may be quite dissimilar to those which occur in albumin. In addition, native albumins are ound in dispersion, whereas gluten occurs as a solid, and the dispersion of the latter in any solvent must change at least its state of aggregation. Furthermore, relatively strong solvents are required to disperse gluten and it is highly probable that these cause greater alterations in this protein than those which occur when albumins are dispersed in water.

As dispersed gluten can be treated quite drastically without evidence of precipitation, measurement of the amount of insoluble material, the usual criterion of alteration, cannot be used to study the changes resulting from treatments. Viscosity measurements, however, present attractive possibilities, since gluten dispersions, in common with other lyophilic colloids, are characterized by a high viscosity which is conditioned by the state of the dispersed phase.

Unfortunately it is difficult to relate the changes in viscosity to a definite alteration in the character of the dispersed material. Neglecting the electroviscous effect, the viscosity of a suspension, or of a true solution of large molecules, is given by the fundamental equation $\eta = \eta_0(1+k\varphi)$ where η_0 is the viscosity of the medium, k a constant, and φ the fraction of the volume occupied by the dispersed material. Empirical equations of higher degree have been developed by other investigators (13) to fit the results obtained with other systems, but all of these show that the viscosity is determined by the quantities given in the above formula. In these equations the constant k has been given different values (18) and as it is assumed to be dependent on the shape of the particles it has been termed a shape factor. Furthermore, for lyophilic colloids, the quantity φ is much larger than the product of the concentration c and the specific volume v of the dispersed phase and may therefore be termed the "equivalent hydrodynamic volume" (13). Similarly, the quotient φ/c is much larger than the specific volume and may be called the "specific hydrodynamic volume". The larger volume may be explained by assuming that the dispersed particles are either greatly hydrated, or interlocked and connected to form a micellar structure (13, 18). Although true hydration is undoubtedly a factor in determining the swelling and aggregation, and consequently the effective size of the dispersed gluten particles, it seems probable that the high viscosity can be attributed mainly to the mechanical immobilization of the dispersion medium by the presence of bulky

aggregates, or by elongated particles that increase the resistance to shear through mutual entanglement.

The electro-viscous effect adds still another complicating factor. Its magnitude is unknown owing to the existing uncertainties as to the values of the effective dielectric constants and the absolute magnitudes of the electrokinetic potentials in the systems under investigation. It is probable, however, that the electro-viscous effect in the neutral solvents is small compared with the effect of the state of aggregation.

It follows from the above reasoning that a change in the shape or size of the particles may alter the viscosity. From an experimental standpoint Freundlich and Ishizaka (9) have shown that the coagulation of aluminium hydroxide sols is accompanied by an increase in viscosity, and have used viscosity determinations to measure the rate of coagulation. Anson and Mirsky (3) and Loughlin and Lewis (16), however, found that the denaturation of egg albumin was accompanied by an increase in viscosity regardless of whether aggregates formed or not.

In spite of the obvious importance of the problem no adequate investigations of solvents, designed to determine which cause the least change in gluten, have been made. It was hoped that in the present study alterations in viscosity could be taken as the chief criterion of change.

2. Methods

Preparation of Dispersions

The flour from which the gluten for the main series of experiments was obtained was milled from a high grade sample of hard red spring wheat (Marquis) and was free from bleaching agents and chemical improvers. In preparing the gluten, 250 gm. of flour was mixed into a firm dough and allowed to stand for 1 hr. in about four times its weight of distilled water, after which most of the starch was removed by kneading under a stream of tap water for 30 min. and finally under distilled water for 10 min.

The dispersing agents originally employed were 0.1 *N* sodium hydroxide, 0.1 *N* acetic acid, 30% urea and 12% sodium salicylate, but supplementary experiments were conducted with other concentrations of the neutral solvents. The authors have reported elsewhere (8) that 24% urea and 8% sodium salicylate are capable of dispersing gluten completely, but the work was begun with the higher concentrations mentioned above because reliable information regarding the minimum concentrations required was not then available. The concentrations of alkali and acid were chosen, after preliminary trials, as giving rates of dispersion within the range limited by the two neutral solvents.

Dispersion was accomplished by placing the wet gluten in the different solvents at 25° C. and shaking vigorously at frequent intervals, as it was found impossible to obtain complete dispersion in urea solution at 0° C. The wet gluten contained about 67% of water and the required amount of solid urea or sodium salicylate was added to obtain the required concentration

of the dispersing agent. No allowance for the water content of the wet gluten was considered necessary with the dilute solutions of acid and alkali employed. As urea solutions tend to become alkaline on standing, these were stabilized at pH 6.8 using Sørensen's phosphate buffers ($KH_2PO_4+Na_2HPO_4$).

The average length of time required to obtain complete dispersion in the various solvents was: in sodium hydroxide, 2.5 hr.; in acetic acid, 1.5 hr.; in urea, 1.0 hr.; and in sodium salicylate, 3.0 hr. All dispersions were passed through a Sharples supercentrifuge at a rate of about 50 ml. per min, in order to remove the starch. As sodium salicylate dispersed the gluten less rapidly than the other solvents, dispersions in this reagent were centrifuged when 4 hr. old, and those in the other solvents when 3 hr. old. In all cases the centrifuged material was starch-free and the loss of protein in the centrifuge was slight.

As dispersions of a definite gluten concentration were required for most experiments, the amount of gluten added originally was slightly in excess of that required to give the desired concentration. After centrifuging, the dispersion was analyzed for protein nitrogen, and then diluted with the solvent to the required concentration.

Nitrogen Determination

The ordinary Kjeldahl method was used to determine the nitrogen in weighed samples of the dispersions in alkali, acid and sodium salicylate. With the urea solutions it was necessary first to precipitate the gluten and, trichloracetic acid failing to give quantitative results, complete precipitation was achieved satisfactorily by a freshly prepared solution containing 50 gm. of tannic acid and 25 ml. of concentrated sulphuric acid per litre (17). Forty ml. of this reagent was poured into a 100 ml. centrifuge tube together with enough distilled water to make the volume 100 ml., after addition of an accurately weighed quantity of gluten dispersion, containing approximately 50 mgm. of protein nitrogen. After stirring thoroughly, the mixture was centrifuged and the liquid decanted. The precipitate was washed three times by adding 5–7 ml. of the tannic acid reagent, triturating, diluting to about 100 ml. while stirring, and then centrifuging and decanting. After the third washing the precipitate was transferred to a Kjeldahl flask.

The method was tested by applying it to dispersions in which the weight of wet gluten and of urea solution were both accurately known and which were not passed through the supercentrifuge. The nitrogen content of the wet gluten having also been determined, it was possible to compare the quantities calculated from the weights of samples used with those found by the tannic acid precipitation method. The results given in Table I show that the method was satisfactory, the maximum difference between the value calculated and that found being 0.10 in 8.32 or 1.2%.

Since some of the dispersions were too viscous to be pipetted accurately, all portions taken for analysis and dilution were weighed and, in consequence, the gluten concentrations are expressed throughout this paper as mgm. of protein nitrogen per gm. of dispersion.

TABLE I

RECOVERY OF PROTEIN NITROGEN FROM GLUTEN DISPERSIONS IN 30% UREA

Nitrogen content of wet gluten		Weight of wet gluten, gm.	Weight of dispersion, gm.	Mgm. of protein N per gm.	
Replicates, %	Average, %			Calculated	Determined
4.27, 4.29, 4.27, 4.38	4.30	9.339	82.280	4.88	4.83
4.26, 4.18, 4.23, 4.44	4.28	6.003	31.934	8.05	8.07
4.32, 4.39, 4.35	4.35	3.931	20.551	8.32	8.22
4.66, 4.59, 4.70, 4.64	4.65	38.85	180.0	10.04	10.02
4.23, 4.26, 4.01, 4.12	4.15	5.361	16.579	13.42	13.32

Viscosity

As the viscosity of such lyophilic colloids as protein dispersions is, in general, dependent on the method of measurement, and particularly on the rate of shear, it is frequently referred to as an "apparent" rather than a "true" value. Comparable results can be obtained only in a carefully standardized type of viscometer in which the rate of shear is approximately the same for dispersions of different viscosity. Ideally this would require a different viscometer for each viscosity but, this being impracticable, the set of standard U-tube viscometers recommended by the British Engineering Standards Association (5) was employed. Although these viscometers are, for several reasons, not ideal for measuring the apparent viscosity of protein dispersions, they possess the advantage of providing a series of standard design, each of which is used over only a limited viscosity range. The results obtained, therefore, can justifiably be expressed in absolute rather than in relative units.

The viscosities of the dispersions studied fell within the ranges of the No. 1 and No. 2 viscometers of the set, namely, 0.9 to 7.2 and 5.4 to 43.0 centipoises respectively. The No. 1 viscometers were standardized at three points within their viscosity range, using water and 20 and 40% sucrose solutions at 25° C., and the No. 2 viscometers at four points, namely, 40% sucrose solution at 20 and 25° C., and 60% sucrose at 25 and 30° C. The sucrose used was chemically pure and had a melting point of 186° C. All temperatures were maintained within 0.02° C. of the stated values, and the densities of the liquids used were determined at the same temperatures using 25 ml. specific gravity bottles with drilled stoppers. In weighing, the usual corrections were made for the buoyancy of air, etc. The viscosity of the solutions used was obtained from the International Critical Tables (12). The results of the standardization showed that the constant applicable to a particular viscometer could be used throughout its entire range, as the variation in this quantity never exceeded 0.5%, and showed no evidence of being systematic.

TABLE II

VISCOSITY OF SOLVENTS

Solvent	Viscosity, centipoises	
	Determined	I.C.T.*
0.1 *N* acetic acid	0.90	—
0.1 *N* sodium hydroxide	0.92	0.92
24% urea	1.15	1.09
30% urea	1.21	1.16
36% urea	1.29	1.26
40% urea	1.34	1.33
30% buffered urea	1.22	—
8% sodium salicylate	1.10	1.09
10% sodium salicylate	1.17	1.16
12% sodium salicylate	1.22	1.23

* *Obtained by graphical interpolation where necessary.*

A further check on the standardization of the viscometers was obtained from measurements made on the dispersing agents. The results obtained are given in Table II, together with the viscosities for these solutions as given in the International Critical Tables. There is good agreement between the two sets of values excepting for urea solutions, for which the determined values fall closer to a straight line than the corresponding values given in the Critical Tables.

The viscosity and density measurements on the gluten dispersions were all made at a temperature of 25.0±0.02° C. Usually only two readings were taken, but if these did not check within 0.2% the determination was repeated.

Salting-out Tests

The method employed in salting out the dispersions in the neutral solvents was to add slowly a definite quantity of magnesium sulphate solution to 10.0 ml. of gluten dispersion. To effect measurable precipitation a 20% solution was required for salting out the gluten from urea solutions, and a 5% solution for dispersions in sodium salicylate. After adding the salt solution the dispersion was shaken, allowed to stand for one hour at room temperature, centrifuged in an ordinary centrifuge, and the precipitate washed with a solution of the same composition as that in which precipitation occurred. The nitrogen content of precipitates from dispersions in sodium salicylate was then determined directly. Precipitates from urea dispersions were redispersed in 30% urea solution and these analyzed for protein nitrogen, using the tannic acid method described above.

Hydrogen Ion Concentration

This determination was made at room temperature using a Clark hydrogen electrode and a calomel electrode containing a saturated potassium chloride solution.

3. Experiments

The first experiments performed were concerned with the reproducibility of the viscosity results, and the effect of gluten concentration on the viscosity of dispersions in all four solvents. Further experiments were then made on the effect of solvent and of hydrogen ion concentration on the viscosity of dispersions in the neutral solvents. The viscosity changes following dispersion were studied during storage at 0 and 25° C. in order to determine whether, and when, a constant viscosity level was reached in each of the reagents. The results of these experiments demonstrated that the viscosity of gluten dispersions, and the changes which occurred during storage, depended on the

solvent, but gave no evidence as to which of the solvents maintained the original properties of the gluten to the highest degree.

Two additional experiments were performed to obtain more definite evidence on this point. In the first of these, gluten dispersed in each of the four solvents was submitted to prolonged storage and then recovered, where possible, by salting out, and examined. In the second experiment, the viscosity of dispersions of gluten of different quality was studied with the object of determining whether or not the difference between the glutens was reflected in the viscosity of their dispersions. Solvents in which the different glutens have different viscosities, if these are related to the quality of the flour, can reasonably be regarded as superior to solvents in which all glutens have the same viscosity, since the latter condition suggests that certain of the original properties of the gluten have been lost.

TABLE III

REPRODUCIBILITY OF THE VISCOSITY OF DISPERSIONS CONTAINING 5 MGM. OF PROTEIN NITROGEN PER GM.

Solvent	Age of dispersion, days	Viscosity, centipoises
0.1 *N* sodium hydroxide	1	1.71
	1	1.92
	1	1.73
0.1 *N* acetic acid	3	1.70
	3	1.76
	3	1.78
30% buffered urea	1	4.12
	1	4.12
	1	4.03
10% sodium salicylate	1	3.47
	1	3.48

Reproducibility

In order to determine whether consistent results could be obtained, a number of gluten dispersions containing 5.00 mgm. of protein nitrogen per gm. of dispersion were prepared and their viscosity measured. These measurements, together with those from certain subsequent experiments, are given in Table III. The viscosity of the dispersions in acid and alkali exhibits the greatest variability. The maximum difference between the different dispersions in the neutral solvents is about 2%.

Effect of Gluten Concentration

In order to reach a decision as to the best concentration of protein to use in subsequent experiments, dispersions containing 4.30, 5.24, 8.00, 9.48 and 10.7 mgm. of protein nitrogen per gm. of dispersion were prepared in 30% urea solution. The viscosity of these dispersions when one day old, taken from the time of placing the wet gluten in the solvent, is plotted against the concentration in Fig. 1. Dispersions of protein concentrations higher than 10.0 mgm. of nitrogen per gm. were found somewhat difficult to handle, so this concentration was chosen as the maximum for use in subsequent experiments with all solvents and a concentration of 5.00 mgm. of protein nitrogen per gm. was used as representative of the more dilute dispersions.

The viscosity-concentration relation in the other solvents was not specifically investigated. However, incidental to the subsequent preparation

of dispersions in these reagents containing 5.00 and 10.0 mgm. of protein nitrogen per gm., a few results were collected on the effect of concentration on viscosity, and these have also been plotted in Fig. 1. It is evident that the viscosity-concentration relation in the neutral solvents is distinctly curvilinear, while in acid and alkali it is almost linear.

The non-linear relation obtained in urea solutions shows definitely that the equation $\eta = \eta_0(1 + k\varphi)$ is not applicable to this system regardless of the value of φ. Kunitz's (14) equation was therefore applied in an expanded form which included the cubic term, and also in its complete form. The equivalent hydrodynamic volume φ and the specific hydrodynamic volume φ/c of one mgm. of protein nitrogen were then computed using both forms of the equation. Both gave essentially constant values for φ/c at different protein concentrations in a given dispersing agent, but those calculated by the cubic equation were larger than those computed by the complete equation. The complete equation was used since Kunitz has shown that it gives values of φ/c which are in agreement with those obtained by other methods. The values of φ and φ/c thus computed are given in Table IV. The curves in Fig. 1 were constructed by substituting the average values of φ/c in Kunitz's equation.

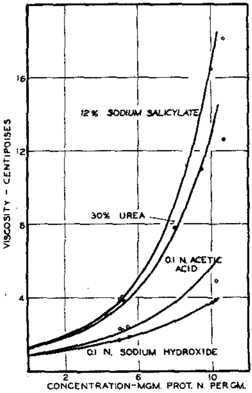

FIG. 1. *Effect of protein concentration on the viscosity of dispersions.*

TABLE IV

EQUIVALENT AND SPECIFIC HYDRODYNAMIC VOLUME OF DISPERSED GLUTEN

Solvent	Concentration, mgm. protein N per gm. (c)	Relative viscosity (η/η_0)	Equivalent hydrodynamic volume (φ)	Specific hydrodynamic volume (φ/c)
0.1 *N* sodium hydroxide	5.00	1.86	0.130	0.026
	5.46	2.00	0.145	0.027
	10.0	4.07	0.275	0.027
	10.2	4.20	0.280	0.027
0.1 *N* acetic acid	5.00	2.54	0.180	0.036
	5.17	2.49	0.175	0.034
	5.46	2.67	0.200	0.037
	10.3	5.40	0.320	0.031
30% urea	4.30	2.50	0.186	0.043
	5.24	3.20	0.232	0.044
	8.00	6.44	0.345	0.043
	9.48	9.09	0.395	0.042
	10.7	10.40	0.415	0.039
12% sodium salicylate	5.00	3.23	0.235	0.047
	5.08	3.29	0.238	0.047
	10.0	13.45	0.452	0.045
	10.7	14.85	0.463	0.043

Effect of Solvent Concentration

In order to study the effect of different concentrations of the solvent on the viscosity of a system having a fixed protein concentration, portions of a dispersion in 30% urea solution were adjusted to various urea concentrations and the same protein concentration, by adding water, 30% urea, or solid urea. All adjustments were made by weight. The final protein concentration was, in all cases, 4.30 mgm. of protein nitrogen per gm. After adjusting the urea concentration, the dispersions were stored at 0° C. for 12 hr. before the viscosity was determined. The results obtained are given in Fig. 2, together with the viscosity of aqueous urea solutions. It is evident that the increase in viscosity with increasing urea concentration is much larger in the presence of protein. This may possibly be explained hydrodynamically by increased mutual interference between the urea molecules and the large protein particles, but the system is too complicated to warrant further discussion.

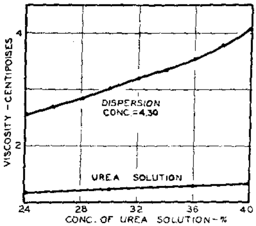

FIG. 2. *Effect of urea concentration on the viscosity of dispersions.*

No special study of the effect of the concentration of sodium salicylate on the viscosity of dispersions was undertaken, but during the course of the investigation, the viscosity of dispersions containing 5.00 mgm. of protein nitrogen per gm. in 8, 10 and 12% sodium salicylate was found to be 3.09, 3.47 and 3.94 centipoises respectively. This again shows the great effect of the concentration of the solvent on the viscosity of the dispersion.

Effect of Hydrogen Ion Concentration

An experiment was made to determine the effect of hydrogen ion concentration on the viscosity and stability of gluten dispersions in the neutral solvents. Portions of an 8-hr.-old dispersion in urea solution were adjusted to different hydrogen ion concentrations by adding buffer solutions, hydrochloric acid, or sodium hydroxide, made up in 30% urea. Sørensen's phosphate buffers were used from pH 6.12 to 8.05, but even within this range some dispersions were also adjusted with dilute acid or alkali in order to secure results comparable with those obtained outside this range. The final protein concentration of the dispersions was, in all cases, adjusted to 5.00 mgm. of protein nitrogen per gm. The hydrogen ion concentration of a 10-hr.-old dispersion in sodium salicylate was adjusted by adding solutions of sodium hydroxide in 10% sodium salicylate, again adjusting the final protein concentration to 5.00 mgm. of protein nitrogen per gm. No effort was made to prepare acidic samples in sodium salicylate owing to the insolubility of salicylic acid in water. All samples were stored at 0° C. after the hydrogen ion concentration was adjusted. As the dispersion medium had a different composition for each hydrogen ion concentration, the viscosity of

the media was determined. This varied from 1.20 to 1.21 centipoises in the urea solutions containing acid and alkali, and from 1.22 to 1.25 centipoises in those containing phosphate buffers. The viscosity of the sodium salicylate media varied from 1.17 to 1.19 centipoises. These small differences in the viscosity of the solvents would have little effect on the results.

It is evident from Fig. 3 that in urea solutions between pH 6.1 and 9.2 the viscosity is independent of the hydrogen ion concentration. On the acid side of this range the viscosity increases markedly with increasing acidity up to pH 3.9, the most acidic condition employed. On the alkaline side the viscosity increases with pH from pH 9.2 to 10.7 where it reaches a maximum and then decreases as the pH increases to 12.4, the most alkaline reaction studied. Moreover, it was found that between pH 3.9 and 6.1 and between pH 9.2 and 12.4 the viscosity decreased so rapidly with time that it was impossible to get consistent results. In determining the viscosity in these ranges, the first measurement was started five minutes after the sample was placed in the viscometer, since this was found to be sufficient time for it to reach 25° C. As each determination required about 10 min. the difference between the first and third reading corresponds to the viscosity decrease over a period of about 20 min. and is represented by the cross-hatched portion between the two curves in Fig. 3. It is evident from this figure that the viscosity fall-back is proportional to the viscosity increase. From the work of Sharp and Gortner (21) it seems highly probable that, had observations been made at pH values below 3.9, the viscosity would have been found to reach a maximum at about pH 3.0 on the acid side and doubtless the viscosity fall-back with time would have been found to be related to the absolute viscosity as in the alkaline region. It should also be noted that since the dispersions had doubtless suffered a viscosity decrease during the preceding storage at 0° C., and a further fall-back while being brought to a temperature of 25° C., even the upper curve by no means represents the maximum viscosity attained by the system.

The relative rate of fall-back at different hydrogen ion concentrations over a period of 24 hr. at 0° C. is shown in Table V by the difference between the observed viscosity of the dispersions when one day and two days old. Between pH 6.1 and 9.8 no significant difference was observed but beyond this range the viscosity decreased. The values given are those obtained from the first observation made on the two successive days. It is evident that there is a close correlation between the viscosity decrease at 0° C. and that observed at 25° C., although comparisons of the magnitude of the decrease

TABLE V

DECREASE IN VISCOSITY OF DISPERSIONS IN 30% UREA DURING STORAGE AT 0° C. AT HYDROGEN ION CONCENTRATIONS OUTSIDE THE STABLE RANGE

pH	Viscosity, centipoises		
	1 day old	2 days old	Decrease
3.88	7.73	7.30	0.43
4.40	6.44	6.09	0.35
5.65	4.89	4.77	0.12
10.2	4.88	4.76	0.12
10.6	5.49	5.18	0.31
11.6	4.21	4.16	0.05

bserved over a 24-hr. period at 0° C., with those obtained over a 20-min. eriod at 25° C., show that the system is much more stable at the lower emperature.

The data plotted in Fig. 3 show that the viscosity of dispersions in 10% sodium salicylate is independent of the hydrogen ion concentration from pH 6.7, the most acid reaction studied, to pH 9.2. More alkaline dispersions increase slightly in viscosity as the pH increases to about 10.0 and then decrease again as the pH increases to 11.4, the most alkaline condition used. At pH values higher than 9.2 these dispersions appear to be more stable than those in urea, since no decrease in viscosity was observed over a 20-min. interval although a longer storage period at 25° C. might have revealed a behavior similar to that observed with dispersions in urea solution.

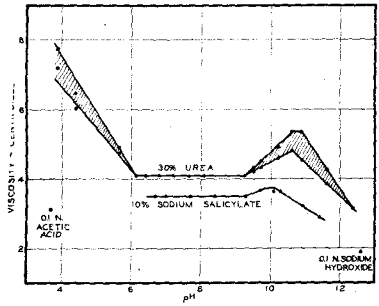

Fig. 3. *Effect of hydrogen ion concentration on the viscosity and stability of dispersions.*

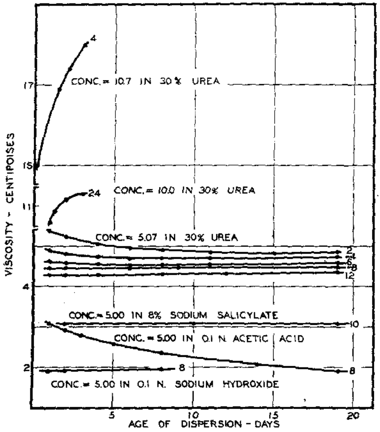

Fig. 4. *Effect of storage at 0° C. on the viscosity of dispersions.*

The viscosity and pH of one-day-old dispersions in sodium hydroxide and acetic acid are also plotted in Fig. 3. The viscosity of gluten in these reagents is not comparable with that obtained in the neutral solvents, but it is interesting to note that the pH of a dispersion in sodium hydroxide is beyond the unstable range on the alkaline side, while the pH of an acetic acid dispersion is probably about the middle of the unstable range on the acid side. This may account for the marked difference in the stability of dispersions in alkali and acid (Figs. 4 and 5), a point which will be discussed in the next section.

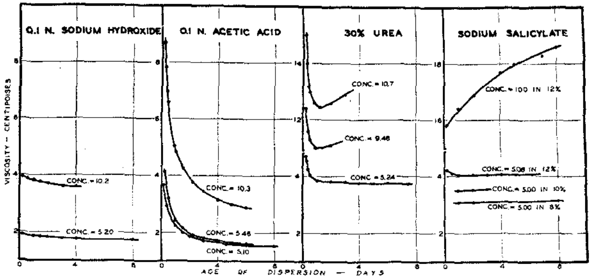

FIG. 5. *Effect of storage at 25° C. on the viscosity of dispersions.*

Changes Following Dispersion

In order to gain some idea of the changes which occur following dispersion, and also to determine the stability of the dispersed material, the viscosity changes occurring during storage at 0 and 25° C. were studied. Since the gluten had to be dispersed sufficiently to permit centrifuging before the viscosity could be measured, it had usually been exposed to the solvent for two hours or more before the first observation was made. The study of the viscosity changes occurring at 0° C. was further complicated by the fact that gluten would not disperse in urea solutions at this temperature. In order to have the results in all solvents comparable it was necessary to effect complete dispersion at 25° C. before placing the dispersions in ice water. Periodic measurements of the pH of the dispersions were made during storage. The maximum change observed was a decrease from pH 12.5 to pH 12.0, which occurred in dispersions in sodium hydroxide during eight days of storage at 25° C. Dispersions in acetic acid and sodium salicylate showed no significant change, while the pH change of dispersions in urea was less than 0.20. It is evident from Fig. 3 that this change could not have had any significant effect on the viscosity.

In the experiment on the effect of storage at 0° C., dispersions in urea solution were studied more extensively than those in other solvents. Both concentrated (10.0 mgm., or more, of protein nitrogen per gm.) and dilute (5.00 mgm. of protein nitrogen per gm.) dispersions were employed, the latter being centrifuged when 1.2 hr. old and portions placed on ice after 2, 4, 6, 8, and 12 hr. of storage at 25° C. in order to study the effect of these treatments. In this experiment the first viscosity measurement was made when the dispersion was one day old.

The viscosity changes with time of storage at 0° C. in all four solvents are shown in Fig. 4, all times being taken from the moment the wet gluten was placed in the dispersing reagent. The protein concentrations, in terms of mgm. of protein nitrogen per gm. of dispersion are given in the figure,

hile the number at the end of each curve gives the number of hours that ıe dispersion was held at 25° C. prior to storage at 0° C. It was found npossible to follow the viscosity changes of a dispersion in alkali for more han eight days, as a slight precipitate invariably formed about this time. t is evident from the figure that the viscosity of the dilute dispersions in the ıeutral solvents is higher than that of dispersions in acid and alkali. In ırea the viscosity decreases with increasing length of the initial storage ›eriod at 25° C. The viscosity of dispersions in sodium hydroxide, sodium ›alicylate and urea, when held initially for a period of six hours or longer ıt 25° C., remains practically constant, while that of dispersions in acetic ıcid decreases throughout the entire period. In contrast to these results ›btained with dilute dispersions, the concentrated dispersions in urea solutions show a marked increase in viscosity during a four-day period at 0° C.

The viscosity changes which occur during storage at 25° C. are shown in Fig. 5. In all cases the time was measured from the moment the gluten was placed in the solvent. Again the viscosity of dilute dispersions in alkali and acid is much lower than of those in the neutral solvents. The viscosity of dispersions in acetic acid shows a marked decrease during eight days of storage at 25° C., the greatest decrease occurring in the first two days. A dilute dispersion in urea also shows a marked viscosity decrease during the first day, after which it remains constant. Dispersions of similar concentration in sodium hydroxide and 12% sodium salicylate show a very slight decrease in viscosity at first, and then remain constant for the rest of the period. Concentrated dispersions in sodium hydroxide and acetic acid decrease in viscosity during the entire period, but the fall-back is much greater in the latter solvent. In urea solutions the concentrated dispersions decrease in viscosity during the first day, but instead of remaining at a constant level, as with dilute dispersions, the viscosity later increases. Concentrated dispersions in sodium salicylate increased in viscosity throughout the period studied.

TABLE VI

PROTEIN NITROGEN SALTED OUT BY MAGNESIUM SULPHATE FROM DILUTE DISPERSIONS STORED AT 25° C.

Solvent	$MgSO_4$ added to 10.0 ml.	Age of dispersion when salt added, hr.	Protein nitrogen precipitated,	
			mgm.	%
30% urea	2.75 ml. of 20%	4	12.2, 14.6	24.4
		46	6.1, 7.4	12.3
8% sodium salicylate	1.50 ml. of 5%	10	15.3, 15.1	27.7
		48	12.5, 12.2	22.5

A number of salting-out tests were conducted on dilute dispersions in the neutral solvents during storage at 25° C. The results given in Table VI show that there was little difference between the amount of gluten salted

out from dispersions in sodium salicylate that were 10 and 48 hr. old. Reference to Fig. 5 suggests that the viscosity decrease over this period was also small. In urea solution the amount salted out after 46 hr. was considerably less than that obtained after 4 hr. and, as there was also a large viscosity decrease during this period (Fig. 5), it appears that the decrease in viscosity is due to a continuation of the dispersing action of the solvent rather than to a dehydration, for, had the latter reaction predominated, it might reasonably be expected that more, rather than less, of the gluten in the older dispersions would be salted out.

Recovery of Gluten from Various Solvents

An attempt was made to recover the gluten by salting out after prolonged dispersion, in the hope of obtaining more definite evidence as to which of the four solvents caused the least change in its character. The dispersions employed contained 5.00 mgm. of protein nitrogen per gm. and had been stored at 25° C. for three weeks. By this time a slight precipitate had already formed in the dispersion in sodium hydroxide. The dispersions were salted out by the addition of magnesium sulphate solution, those in acid and alkali having been previously neutralized.

The dispersion in sodium hydroxide became cloudier on adding the salt solution but no additional precipitate formed, nor could that which had previously appeared be matted into a gluten ball. Those in acetic acid and urea yielded a precipitate which was recovered by centrifuging and decanting the liquid. No matter how carefully the precipitate from the dispersion in acetic acid was handled it was impossible to mat it into a gluten. The precipitate from the dispersion in urea matted to form a gluten which was smooth, but inelastic and lacking in resilience. The dispersion in sodium salicylate yielded a gluten which came down without centrifuging and was smooth, but tough and inelastic. These observations indicate that the solvents fall in the following order with respect to decreasing effect on the protein: sodium hydroxide, acetic acid, urea, and sodium salicylate.

Viscosity of Glutens from Different Flours

A further comparison of the effect of the various solvents on gluten was made by studying the viscosity of dispersions of several glutens of widely different quality. The flours used in the first experiment included one of high baking strength milled from a sample of Marquis wheat, one of intermediate strength milled from Garnet, and a weak commercial pastry flour. All of the dispersions were centrifuged when six hours old, and analyzed. When from 9–11 hr. old, they were diluted to 5.00 mgm. of protein nitrogen per gm. A temperature of 25° C. was maintained during preparation and storage. The viscosity was determined when they were 1, 2, 3, and, in some cases, 4 days old.

The results given in Table VII show that the dispersions of the three glutens in urea solution had different viscosities, which remained practically constant over a three-day period, and which were correlated to some extent

TABLE VII

VISCOSITY OF DISPERSIONS OF GLUTEN OBTAINED FROM FLOURS OF DIFFERENT BAKING STRENGTH

Solvent	Flour	Viscosity, centipoises			
		1 day old	2 days old	3 days old	4 days old
0.1 *N* sodium hydroxide	Marquis A	1.73	1.69	1.68	
	Garnet	1.74	1.71	1.68	
	Commercial pastry	1.70	1.67	1.66	
0.1 *N* acetic acid	Marquis A	2.29	1.92	1.78	1.71
	Garnet	1.97	1.74	1.66	1.60
	Commercial pastry	2.16	1.85	1.73	1.66
30% buffered urea	Marquis A	4.04	4.06	4.04	
	Garnet	3.27	3.23	3.22	
	Commercial pastry	3.19	3.09	3.09	
10% sodium salicylate	Marquis A	3.47	3.51	3.59	
	Garnet	2.83	2.86	2.93	
	Commercial pastry	2.93	2.91	2.95	

with baking strength. The gluten from the strong Marquis flour exhibited a significantly higher viscosity in sodium salicylate than did glutens from the other flours. These had about the same viscosity when three days old, although the gluten from the Garnet flour had the lowest viscosity on the first day. This suggests that glutens retain their individuality to a lesser degree in sodium salicylate than in urea solution. The viscosity of all of the dispersions in sodium hydroxide was essentially the same. In acetic acid the three dispersions differed in viscosity when one day old, but on the fourth day, when the viscosity fall-back was practically complete, all had essentially the same viscosity. It is concluded therefore that dispersion in the neutral solvents caused less drastic changes in the original gluten than dispersions in acid and alkali.

Since the viscosity of gluten dispersed in urea solutions, and to a lesser extent that of gluten dispersed in sodium salicylate solutions, decreases with the baking strength, it appears that these solvents are not capable of dispersing a strong gluten to the same extent as a weak one. Although a study of gluten quality was not contemplated in this investigation, the foregoing results suggested that the viscosity of a dispersion in a neutral solvent might be used as a measure of quality. In order to study the point further the gluten from four flours, which differed less in baking strength than those previously used, was dispersed in the two neutral solvents and the viscosity of the dispersions determined as before. The results obtained, together with the protein content of the wheat from which the flour was milled and the loaf volume and texture score resulting from an experimental baking test, are given in Table VIII.

The actual baking quality as judged by the loaf volume, places the flours in the order in which they are listed. It cannot be said, however, that these

TABLE VIII

VISCOSITY OF GLUTEN DISPERSED IN THE NEUTRAL SOLVENTS IN RELATION TO THE BAKING STRENGTH OF FLOUR

Flour	Protein in wheat[1], %	Baking test by bromate formula[2]		Viscosity, centipoises					
		Loaf volume, ml.	Texture score[3]	In 30% urea			In 10% sodium salicylate		
				1 day	2 days	3 days	1 day	2 days	3 days
1	15.3	687	7.5	3.38	3.30	3.30	2.98	2.99	3.02
2	14.8	648	5	3.35	3.29	3.25	2.99	2.94	2.94
3	15.4	616	3	3.26	3.12	3.08	3.00	3.00	3.02
4	16.0	588	5.5	3.22	3.15	3.08	2.90	2.84	2.87

[1] *Expressed on a 13.5% water basis.*
[2] *100 gm. of flour on a 13.5% water basis.*
[3] *Perfect score = 10.*

differences in baking quality are due entirely to the quality of the gluten. The protein content of Flour 2, for instance, is lower, and that of Flour 4 higher, than those of Flours 1 and 3. Furthermore, such factors as diastatic activity, etc., which were not determined in the flours studied, also affect the loaf volume. The viscosity of the gluten from the last two flours was lower than that from the first two, when dispersed in urea solutions, while the viscosity of the last flour was lower than that of the others in sodium salicylate solution. Determination of the viscosity of gluten in a neutral solvent would thus seem to be of doubtful value for predicting the quality of the protein. The technique is rather time-consuming and laborious, and involves certain processes, such as gluten washing, which are difficult to standardize rigidly when the glutens vary in strength. Nevertheless, this type of viscosity determination appears preferable to those performed on acidulated flour-in-water suspensions. The complicating effects introduced by the presence of starch, electrolytes, and varying protein concentrations are removed on washing the gluten and adjusting the protein concentration after dispersion, and in addition, the viscosity is measured at a pH at which the protein is reasonably stable.

4. Discussion of Results

It has been shown that the viscosity of gluten dispersions in urea and sodium salicylate is much higher than that of dispersions of the same concentration in alkali or acid. This is attributable in part to the higher viscosity of the solvents themselves, but the application of Kunitz's equation to the relative viscosity of the dispersions shows that the equivalent hydrodynamic volume of the gluten is larger in the neutral solvents than in acid or alkali. This indicates either a greater solvation or a lower degree of dispersion in the former solvents. The latter seems more probable.

The changes in the viscosity with hydrogen ion concentration must represent some change in the state of the dispersed gluten. It seems unlikely

that either the electro-viscous effect, or differences in solvation alone could account for the nature and magnitude of these. The high unstable viscosity maxima may, however, reasonably be attributed to an extension and swelling of the particles preceding their further dispersion into the smaller units which constitute the stable system at extreme hydrogen ion concentrations.

These results are of interest in connection with viscosity tests of acidulated flour-in-water suspensions. Since Sharp and Gortner's (21) work many investigators have tried, with varying degrees of success, to use this test as an index of protein quality. Difficulty has been experienced in standardizing the procedure rigidly enough to give reproducible results, although Reiman (20) found that with proper precautions, particularly with respect to the lactic acid, results could be duplicated. The present work shows that the region of maximum viscosity is also the region of maximum instability and probably explains the difficulty of obtaining consistent results. Measurements at lower temperatures, however, where the proteins are more stable, may prove more satisfactory.

The viscosity changes which occur during storage at 0° C. and 25° C. give some idea of the time required for complete dispersion, and the stability of the resulting system. The relatively constant viscosity values obtained in sodium hydroxide suggest that the dispersing action of this solvent is extremely rapid, and that it is practically complete before an observation can be made. Acetic acid appears to disperse the gluten more slowly and a constant viscosity level was never reached. The behavior of concentrated dispersions in the neutral solvents indicates that the initial dispersion reaction is followed by some form of aggregation or flocculation. This second type of reaction was never evident in dilute dispersions in any of the solvents or in the concentrated dispersions in acid and alkali.

In urea solutions the viscosity level reached by dilute dispersions was considerably higher at 0° C. than at 25° C. It seems likely that this can be attributed largely to increased dispersion at 25° C. rather than to increased solvation at the lower temperature, especially since the fall-back at 25° C. appears to be irreversible, there being little tendency for the viscosity to increase on storage at 0° C. after storage at 25° C. Furthermore, the dispersion is more resistant to salting out after storage at 25° C. than it was originally, a condition scarcely compatible with a more dehydrated state of the gluten particles. The higher viscosity observed at 0° C. consequently indicates that the degree of dispersion in urea solutions is dependent on the temperature.

Although these experiments give considerable information regarding the changes which occur in the different solvents during dispersion, they provide no conclusive evidence as to which is the best solvent for gluten. If McBain's (18) criterion, that the best solvent is the one giving the least viscous dispersion, is accepted, then sodium hydroxide and acetic acid are superior to the neutral solvents. This criterion, based mainly on colloidal considerations, however, is scarcely valid for protein dispersions where, quite apart from the

degree of dispersion, irreversible physical and chemical changes may be brought about by the solvent. On the other hand, if the properties of gluten are determined to some extent by its state of aggregation, the dispersions in solvents causing a low degree of dispersion, as shown by a high viscosity, may be expected to represent the properties of the original substance more closely than those in solvents causing a high degree of dispersion. If this reasoning is correct, then the neutral solvents are better than acid or alkali. As an increase in viscosity occurred in concentrated dispersions in the neutral solvents, it may be contended that this evidence of coagulation marks them as inferior. This, however, is not a valid criticism since an alteration in the proteins, such as denaturation, may occur in alkali and acid, although coagulation may be prevented.

It is concluded from the experiments on gluten recovery, and on the viscosity of gluten from flours differing in baking strength, that dispersion in neutral solvents alters the gluten less than dispersion in acid or alkali. The main difference between the two classes of solvents appears to be that alkali and acid effect a higher degree of dispersion. Whether or not this is accompanied by a chemical change is unknown, but gluten after dispersion in alkali or acid appears to have lost its original properties, whether judged by the salted-out product or by the viscosity behavior of a series of glutens known to have differed originally.

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ROOT ROT OF GINSENG IN ONTARIO CAUSED BY MEMBERS OF THE GENUS *RAMULARIA*[1]

BY A. A. HILDEBRAND[2]

Abstract

Two destructive diseases of the root of ginseng, *Panax quinquefolium* L., have assumed economic importance in the ginseng-growing districts of Ontario. The more destructive of the two diseases, commonly known as the disappearing-rot, is characterized by the fact that affected roots may, in a relatively short time, either completely disappear in the soil or leave as evidence of their presence only a peridermal shell enclosing fragments of vascular tissue. The disease, which is non-systemic, is caused by at least three representatives of the genus *Ramularia* of the Fungi Imperfecti, one of which has been identified as *Ramularia panacicola* Zins., and the other two being new to science, are described as new species for which are proposed the binomials, *Ramularia mors-panacis* and *Ramularia robusta*. No sexual stage has been observed in connection with the three species. They appear to persist in the soil indefinitely either saprophytically or in a dormant condition.

The name rust has been applied to the other serious but less destructive disease because of the occurrence on the surface of affected roots of superficial, rust-colored lesions. The cause of the rust disease has not been definitely established but the evidence thus far suggests that it is probably also caused by representatives of the genus *Ramularia*.

Rotation and rigid sanitation are the only control measures suggested, resistant varieties not having been encountered.

In the present work a large number of additional representatives of the genus, different from each other and from the isolants from ginseng, have been obtained from tissue isolations from similar lesions on various other hosts. Cross-inoculations indicate variations in pathogenicity among the isolants and specificity in host relations. This demonstration of the universal occurrence and parasitic capabilities of soil-inhabiting representatives of the genus *Ramularia* changes the concept of the genus and adds a most important member to the group of facultative parasites associated with root troubles of plants.

Introduction

During recent years growers of American ginseng, *Panax quinquefolium* L., in various parts of Ontario, have found it increasingly difficult to bring to maturity a plant whose root, which is the article of commerce, requires at least five years' growth before it reaches a desirable marketable stage. Of various diseases which are primarily responsible both for impairment of quality and for reduction in yield, two which attack the roots are pre-eminent. The more serious of the two diseases is variously known as the brown rot, the disappearing-rot or, more simply, the rot, while to the other disease has been ascribed the name "rust". The increasing prevalence of one or of both of these diseases in the ginseng-growing districts of Ontario has created a problem of considerable economic importance. The present paper reports the results of investigations carried out in an attempt to discover (i) the cause of the diseases, (ii) the phenomena associated with their occurrence and spread, and (iii) successful methods of control.

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Review of Literature

Diseases of ginseng have been the subject of scientific research since 1904, when Van Hook (10) published the results of investigations carried out in New York State, where within little more than a decade after the plant had been brought under cultivation, diseases had already become serious in almost every ginseng-growing section. Among other diseases Van Hook mentions an end rot of seedlings characterized by a rotting away of the taproot, beginning usually at the lower end. The cause of this disease was not determined and only a very meagre description is appended. Whetzel and Rankin (17) in 1909, and Whetzel and Osner (18) in 1910, having investigated a disease of older roots, which had become widespread and destructive not only in New York but in other ginseng-growing states, and to which had been ascribed the name "rust" or "fibre-rot", concluded that the disease was "but another form of the end rot of seedlings". Whetzel and Rosenbaum (19) in 1912, reaffirmed the above conclusion with regard to this disease, stating after giving a detailed description of symptoms as they appeared on seedlings and on older roots, that "while the absolute proof of the common cause of these different symptoms on seedlings and older roots has not yet been established, the evidence thus far accumulated indicates that they are one and the same thing and may be, at least for the present, referred to as the rust". They also considered the evidence at this time as pointing to the fungus *Thielavia basicola* (B. and Br.) Zopf., as the causal organism of the disease. By 1916 the brown rot or rust was prevalent throughout the ginseng-growing districts of Wisconsin, and Brann (2) records attempts at control by steam sterilization of soil. According to Whetzel, Rosenbaum, Brann and McClintock (20) 1916, all efforts to find a definite organism associated with the disease had failed, and the opinion was expressed that "doubtless several of the root rots (especially those caused by *Alternaria panax* and *Thielavia basicola*) are commonly included with other brown rots and rusty discolorations of the roots under the name rust". Previous to the work of Zinssmeister (25) in 1918, therefore, the term rust had been applied more or less indiscriminately to diseases which, though they may have resembled one another in their general symptomatology, were undoubtedly caused by different organisms. Zinssmeister for the first time employed the term rust to specify a definite root disease. Isolations made from material received from two widely separated states, New York and Wisconsin, yielded in the majority of cases, isolants of *Ramularia*. The latter were resolved into two species which, being new to science, were described and named, one being designated *Ramularia destructans*, the other *Ramularia panacicola*. Both species were found to be pathogenic on dormant roots and evidence was adduced to show that the disease could develop during the dormant season.

Berkeley (1) in 1927, found evidence of the presence of two troubles in certain of the ginseng-growing districts in Ontario; one, of the nature of a soft rot, the other, of a scabby or rusted condition of the roots. Isolations from diseased material yielded several organisms including *Ramularia* spp.

Nakata and Takimota (5) in 1923, published the results of studies of diseases of Asiatic ginseng *Panax ginseng*, in Korea, where the production of this crop assumes an important place in Korean agriculture. Two diseases of the root, red rot and amber-colored rot, both of bacterial origin, are of outstanding importance, being prevalent in all regions of Korea where ginseng is cultivated. Whetzel (21) as recently as 1928 seemed to hold the opinion that the rust in America as defined by Zinssmeister and shown by him to be of fungous origin, is the same disease as the Korean red rot, which, according to the Japanese investigators mentioned above, is a bacterial disease.

Symptoms of the Diseases

Rot

The rot affects directly the parts of the plant that are underground, namely, (i) the root, (ii) the perennial stem, and (iii) that portion of the current-year stem extending upwards from the point of attachment on the perennial stem to the ground level. The most striking indications that the disease is active in a given stand are, first, the failure of the plants to come up in the spring, and second, the wilting of the aerial parts during the growing season. The appearance of a root affected with rot depends on the stage of development of the disease. In the early stages of infection there appear on the root at any point on its surface, either on the main taproot or on the laterals, small, brownish, discolored areas (Plate 1, Fig. 1). At this stage there is no rupturing or distortion of the periderm other than a slight wrinkling, and if the root is cut across, the lesion is found to be quite shallow. The lesions increase rapidly both in surface extent and in depth of penetration (Plate 1, Figs. 2, 3 and 4). If the periderm is removed, the underlying, reddish-brown, diseased tissue is found to be spongy and moist but not water-soaked. Even in advanced stages, the tissues of the root are not resolved into the water-soaked, amorphous masses typical of soft rots. In later stages the perennial stem and the crown of the root may be completely rotted away. This is almost invariably indicated by the ease with which the now wilted stem can be detached. In other cases the root fibres and smaller laterals may have completely disappeared, or, very often, the lower part of the main root itself, or that of one of its larger laterals, may be rotted off, the combined effect being to leave the root in a bare and stubby condition (Plate 1, Fig. 2). In the final stages, very often all that remains of the root are remnants of the peridermal shell enclosing a few fragments of vascular tissue. Finally not a trace of the root is to be found. There does not seem to be any correlation between age and susceptibility to attack, the disease having been observed in seedlings and in roots of all ages up to six years.

Wilting of aboveground parts is sooner or later an indication of a rotted condition of the roots. The extent to which a root may be rotted before the sudden collapse of the aerial parts which have given no evidence of the presence of the disease, is remarkable. In the warmer and drier part of the growing season, plants with rotted roots may wilt during the hottest part of the day and recover towards evening. Such recoveries, however, are only

temporary and soon the wilting becomes permanent. Sometimes, in older plants, one of the compound leaves, three of which arise in a whorl from the apex of the slender, upright stem, will show signs of wilting while the other two appear perfectly healthy. Examination of the root in such cases very often shows that a main branch has rotted away, thus cutting off the water supply from one side of the plant with consequent wilting of the parts more directly affected. Changes in color from the normal dark green of the healthy foliage sometimes accompany wilting but, since similar color changes often result from causes other than the rot, they are of uncertain diagnostic value.

Rust

The appearance of rusted roots also depends on the stage of development of the disease. In earlier stages discolored areas of varying size are to be observed on the surface of the root. These are due to the presence of more or less densely aggregated, slightly raised, reddish-brown spots, which, in turn, vary in size from minute dots whose individuality is only discernible with the aid of a hand lens, to spots of macroscopic size. The latter are irregular in shape and are often extended around the circumference of the root, seeming to follow the depressions of the circular wrinkles which characterize the surface of ginseng roots. At this stage, whatever the size of the individual spots comprising a diseased area, they are quite superficial and, being slightly raised, give to the surface of the affected area a slightly roughened appearance. In later stages, the smaller spots grow larger and coalesce to form definite and continuous lesions, which, though they may involve considerable surface area of the root, penetrate to the depth of a few cell layers only. Owing to the rupturing and sloughing-off of the periderm over considerable areas, the roots at this stage present a decidedly scurfy appearance (Plate 1, Fig. 5). When scraped slightly with a knife blade or even rubbed with the thumb, the diseased tissue is easily detached, leaving exposed the white, healthy tissue beneath. While rust lesions, as in the case of those of the rot, may be found on any part of the root, they appear to be of more common occurrence in the region of the crown, and often extend upwards to involve the perennial stem.

Plants whose roots are badly rusted are often slightly stunted, and mature earlier than those whose roots are healthy. These are almost the only indications of the presence of the disease so far as the aboveground parts are concerned.

Economic Importance and Distribution of the Disease

In the vicinity of Waterford, Norfolk County, where ginseng growing is carried on more extensively than elsewhere in Ontario, the disappearing-rot has caused greater loss than any other disease. From small centres of infection which appear variously and unexplainably scattered throughout the gardens, the disease spreads radially outward, occasioning complete destruction of roots as it advances. Probably one of the chief factors in the rapid spread of the disease, especially in the case of older roots, is the close proximity of the latter to one another, their fibres intertwining in the soil

to form a more or less continuous host-bridge for the spread of the pathogen. Once the soil has become infested, it cannot be sown to ginseng again for an indefinite period and many gardens have had to be abandoned on account of ginseng-sick soil. Although less destructive than the rot, in that it does not occasion the complete disappearance of the root, nevertheless, by rendering large quantities of roots marketable only as culls, the rust also causes heavy losses in certain of the gardens of Norfolk County.

In the parts of Peel and Dufferin Counties which rank next in importance as ginseng-producing centres, a survey has shown that the rust, but not the rot, is prevalent in certain gardens where it is occasioning considerable losses.

Both diseases have been found in widely different types of soil ranging in texture from heavy clay loam, through various intergrades of loam and humus content, to light, sandy loam. Once established, both diseases are serious, seemingly regardless of type of soil or meteorological conditions.

Isolations from Diseased Roots

Rotted Roots

Isolations from diseased roots were made over a period of four years both from material collected personally and from specimens forwarded to the laboratory from various parts of Ontario. In the case of rotted roots showing early stages of infection, a portion of the root showing a small lesion was excised and washed under running water for from 5 to 15 min., a small, fairly stiff-bristled brush being used to facilitate the removal of extraneous material. After final rinsing in sterile water, small bits of tissue were removed from the periphery of the lesion, and transferred to tubed slants. Roots showing more advanced stages of the rot were first washed under running water and then surface-sterilized by immersion in mercuric chloride (1/1000) for periods varying from one to four minutes, depending on the depth of the lesion. After rinsing finally in sterile water the root was cut across through the lesion and from the innermost depth of the latter, where diseased and healthy tissue merged into one another, bits of tissue were removed and transferred to tubed slants. In the case of the more severely diseased roots, macerations of diseased tissue were made in sterile water, after washing and surface sterilization. The suspension was then streaked on plates of solid medium. Potato-dextrose agar acidified to the extent of 2 drops of 25% lactic acid per 15 cc. of medium was employed throughout.

Many different organisms were obtained from rotted roots. Of most frequent occurrence in the aggregate were isolants of *Ramularia*, some of which appeared early in the investigations, others comparatively recently, but all of which were resolved into five different strains or growth forms, for convenience tentatively designated as strains *P*, *M*, *R*, *S* and *B*. The roots from which strains *P* and *M* were isolated were obtained from the same garden, while strain *R* was isolated from roots obtained in another garden about a mile distant. Strains *S* and *B* were isolated from diseased specimens forwarded to the laboratory from two widely separated districts in Ontario.

Second in frequency of occurrence, and in some series of isolations predominating numerically, were different strains of *Fusarium*. Certain of these,

as well as the five strains of *Ramularia*, were obtained in pure culture for subsequent tests as to their pathogenicity. More or less sporadically all through the investigations there also appeared in culture representatives of the genera of fungi so frequently reported in studies of this kind, namely, *Trichothecium*, *Penicillium*, *Aspergillus*, *Verticillium*, *Alternaria* and *Mucor* spp.

Rusted Roots

Rusted roots were much more difficult to work with than rotted roots. The porous and superficial nature of the lesions and the ease with which the infected tissues became detached, rendered it almost impossible either to surface-sterilize the roots or to wash them sufficiently to remove more than the coarser soil particles. The roots were washed as thoroughly as possible through successive changes of sterile water, and transfers of diseased tissue were made to acidified potato-dextrose agar. By this method certain fungi were found consistently enough in association with rust lesions to suggest a possible causal relation. These included five different strains of *Ramularia* (none of which was identical with any of those isolated from rotted roots), several different strains of *Fusarium*, and one species each of *Sporotrichum* and *Hormodendrum*. Pure cultures of all these organisms were obtained by the monospore method. As was the case in plantings from rotted roots, numerous other presumably saprophytic soil-inhabiting fungi appeared in isolations from rusted roots.

Infection Experiments

Several attempts were made to grow ginseng in experimental plots at the laboratory at St. Catharines but with indifferent success, consequently the infection experiments for the most part were carried out in the gardens at Waterford, Norfolk County, where plants of all ages were made available through the co-operation of several growers of the district.

Series A. Involving Organisms Isolated from Rotted Roots

Experiment 1, 1929-30, Garden A, Waterford

Using pure cultures of *Ramularia M*, and two strains of *Fusarium*, *F* and *D*, the three organisms which up to that time had been most consistently isolated from rotted roots, the first infection experiment was carried out, Sept. 5, 1929, in a bed of apparently healthy four-year-old roots growing in heavy, clay-loam soil. Ninety carefully selected roots were involved, 15 being inoculated with *Ramularia M*, 15 with *Fusarium D*, 15 with *Fusarium F*, 15 with a mixture of cultures of *Ramularia M* and *Fusarium D*, 15 with a mixture of cultures of *Ramularia M* and *Fusarium F*, while 15 served as checks. In 8 of the roots in each group of 15, the inoculum was inserted into artificial injuries. The remaining 7 roots, without injury, were sprayed with spore suspensions of the different organisms.

The results of the experiment became known May 21, 1930. The 45 roots which had been inoculated with *Ramularia M* either alone or in combination with each of the two strains of *Fusarium*, had disintegrated to the extent

that only fragments of peridermal shell could be found. Of the 30 roots inoculated with the two strains of *Fusarium* alone, 7 could not be found and 6 others showed various stages of rot. Three of the latter were roots which had been injured when inoculated and it was noted that the rot had not started at the point of injury. The remaining 17 roots had remained healthy except that several of those which had been injured showed at the point of injury small but definite diseased areas, none of which, however, were typical disappearing-rot lesions. Nine of the 15 checks were found to have remained healthy but the remaining 6 had badly rotted roots. The latter were all adjacent to the part of the bed which had not been used in the experiment and subsequent investigation showed that many of the plants in this part of the bed had rotted roots. Specimens of these were taken to the laboratory and from them was isolated not only *Ramularia M* but, in addition, a new and closely related representative of the same genus which was designated *Ramularia P*.

The almost complete disappearance of all roots which had been inoculated with *Ramularia M* either alone or in combination with the two strains of *Fusarium*, as contrasted with the survival of the majority of those which had been inoculated with the *Fusarium* strains alone, strongly suggested *Ramularia M* as a possible causal agent of the disease. Indications were also given that the pathogen was not a wound parasite and that the disease was capable of rapid development at relatively low soil temperatures. At the time the experiment was begun, in Sept., 1929, not one of the 90 roots involved showed any signs of disease. In many cases, however, since only a small area of the root was exposed, it is quite possible that, on many of them, incipient infections were already present on parts not uncovered. In view of our later knowledge of the rapidity with which the disease can develop and spread, it seems equally possible that infections could have taken place after the commencement of the experiment. In any case, the appearance of disease in the check plants, together with the impossibility of completing the rules of proof in the case of the suspected organism, made it necessary to repeat the experiment.

Experiment 2, 1930, Gardens A and B, Waterford

A second infection experiment was carried out in the field June 17, 1930, in two gardens about a mile apart where the soils were of remarkably different texture and consistency, that in garden A—where the first experiment had been carried out—being a heavy clay loam, that in garden B consisting of an easily workable, light loam with high humus content and closely resembling forest soil in appearance and texture. The plants in garden A were five years old, those in garden B, three. The organisms used as inoculum were *Ramularia M*, the more recently isolated *Ramularia P* and two strains of *Fusarium F*, which had been used in the first experiment, and a new strain, *H*, which had meanwhile appeared consistently in isolations from rotted roots. In this experiment inoculation by spore suspension was omitted, all the roots being injured and the inoculum inserted into the injury. The data in connection

with this experiment and the results as observed Aug. 18, 1930, 52 days after inoculation, are summarized in Table I.

TABLE I

SUMMARY OF RESULTS OF INFECTION EXPERIMENT 2, JUNE 17–AUG. 18, 1930

Garden	Age of roots, years	Soil type	Organism		No. roots injured and inoculated	Injured checks	Results
			Fusarium	*Ramularia*			
A	5	Heavy clay loam	- - - -	Strain *P*	16	- - - -	100% disappearing-rot infection
A	5	Heavy clay loam	Strain *H*	- - - -	16	- - - -	100% typical *Fusarium* infection
A	5	Heavy clay loam	- - - -	- - - -	- - - -	12	73% healthy
B	3	Light loam	- - - -	Strain *M*	16	- - - -	100% disappearing-rot infection
B	3	Light loam	Strain *F*	- - - -	16	- - - -	100% typical *Fusarium* infection
B	3	Light loam	- - - -	- - - -	- - - -	12	83% healthy

On Aug. 18, all 16 roots in garden A, which had been inoculated with *Ramularia P* were found to be typically rotted, the majority of them so severely that it was difficult to remove them intact from the soil. Practically identical results were obtained with *Ramularia M* in garden B. Re-isolations from roots from both gardens were disappointing in that cultures of *Fusarium* predominated over those of *Ramularia P* and *Ramularia M* in the ratio of five to three.

The results obtained in the case of roots inoculated with *Fusarium H* (garden A), and *Fusarium F* (garden B), closely approximated one another. In every case small but definite lesions developed around the point of injury, but these lesions differed so markedly from those typical of the disappearing-rot as to be clearly distinguishable from them.

Of the 24 check plants, three in garden A and two in garden B developed typical rot before the termination of the experiment. The rest remained healthy.

From the results obtained in this experiment it appeared that both *Ramularia P* and *Ramularia M*, when inoculated into artificially injured but otherwise healthy ginseng roots, were capable of producing symptoms as characteristic of the disappearing-rot as those resulting from natural infection. Apparently also the disease can develop rapidly in soils differing markedly in texture and content, under the relatively drier and warmer conditions which obtain in midsummer. The two strains of *Fusarium*, *F* and *H*, though slightly pathogenic, produced symptoms entirely different from those of the disappearing-rot. The predominance of strains of *Fusarium* over both *Ramularia P* and *Ramularia M* in isolations from lesions induced primarily by the latter organisms is most reasonably explainable on the basis of secondary invasion by a ubiquitous, saprophytic, soil fungus.

Experiment 3, 1930, Garden B, Waterford

The results obtained in the first two infection experiments had proved almost conclusively that the two forms of *Ramularia*, *P* and *M*, bore a causal relation to the disease, yet, in neither case, had the requirements of the rules of proof been fully satisfied. In the first experiment, re-isolations could not be made from inoculated roots because of the disappearance of the latter before the termination of the experiment. In the second experiment, although the two forms of *Ramularia* were recovered from inoculated roots, *Fusarium* spp. appeared in the majority of the re-isolations. A third experiment was undertaken with slight modifications in the technique which, it was thought, might render more certain the recovery of the primary causal organisms from inoculated roots. In the first two experiments, in cases where roots had been injured and inoculated, a small, moist pad of absorbent cotton was applied to cover the wound after the inoculum had been inserted. In the third experiment this procedure was repeated but, in addition, the smaller pad was covered with a second and larger one of dry non-absorbent cotton, which was pushed well down alongside the root. In this experiment uninjured roots also were inoculated with spore suspensions of the organisms. Sufficient soil was removed to expose a part of the root surface, a heavy spore suspension was applied by means of an atomizer and the soil was replaced. The organisms used were the two forms of *Ramularia*, *P* and *M*, and a new strain of *Fusarium*, designated *Fusarium* 148, which had been obtained in a high percentage of cases from roots inoculated with the two forms of *Ramularia* in Experiment 2. The complete data in connection with this experiment, beginning Aug. 18 and terminating Sept. 19, 32 days later, are summarized in Table II.

TABLE II

SUMMARY OF RESULTS OF INFECTION EXPERIMENT 3, AUG. 18–SEPT. 19, 1930

Organism	No. plants inoculated		Results of inoculation	Results of re-isolations
	Spore susp.	Injured		
Ramularia P	- - - -	8	100% disappearing-rot infection	*Ramularia P*, 61%; *Fusarium* spp. 39%
Ramularia P	8	- - - -	87% disappearing-rot infection	*Ramularia P*, 100%
Ramularia M	- - - -	8	100% disappearing-rot infection	*Ramularia M*, 85%; *Fusarium* spp. 15%
Ramularia M	6	- - - -	100% disappearing-rot infection	*Ramularia M*, 78%; *Fusarium* spp. 22%
Fusarium 148	- - - -	8	50% typical *Fusarium* infection	*Fusarium* 148, 100%
Fusarium 148	8	- - - -	100% healthy	
Checks	- - - -	6	100% healthy	
Checks	6	- - - -	100% healthy	

All 16 roots injured and inoculated with the two forms of *Ramularia*, showed typical disappearing-rot lesions in the region of injury 32 days after inoculation (Text-fig. 1). In the case of the roots inoculated with *Ramularia P*, the organism was recovered from 60.9% of the plantings as compared with *Fusarium* spp. in 39.1%. From the roots inoculated with *Ramularia M*, the organism was recovered in 85% of the plantings, while *Fusarium* spp. appeared

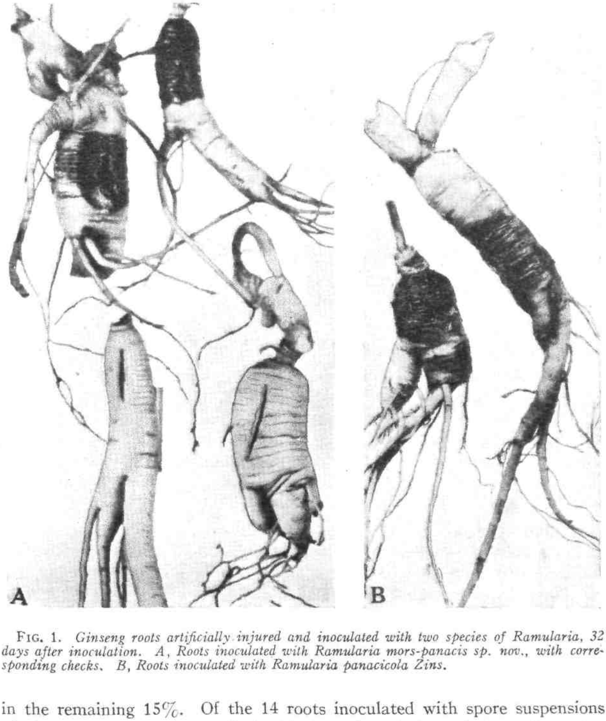

FIG. 1. *Ginseng roots artificially injured and inoculated with two species of Ramularia, 32 days after inoculation. A, Roots inoculated with Ramularia mors-panacis sp. nov., with corresponding checks. B, Roots inoculated with Ramularia panacicola Zins.*

in the remaining 15%. Of the 14 roots inoculated with spore suspensions of the two isolants of *Ramularia*, 13 showed typical rot lesions of varying size, none of which, however, penetrated as deeply into the tissues of the root as did those resulting from wound inoculations, but from all of which, as reference to Table II will show, the organisms were recovered.

Of the 16 roots inoculated with *Fusarium* 148, only four of the eight roots which had been injured developed lesions. These were confined to the immediate region of the injury and differed markedly from typical rot lesions. The roots to which the spore suspension of this organism had been applied remained healthy, as did all the checks.

The results of this third experiment leave little doubt as to the aggressiveness of the two forms of *Ramularia*, *P* and *M*, whether or not an infection court is provided by artificial means. Both strains are extremely virulent in their attack. Although undoubtedly the primary parasites, they are probably not alone responsible for the rapid disintegration and ultimate disappearance of the root. Metabiosis or succession of organisms undoubtedly plays an important part throughout the later phases of the disease.

Fusarium 148 is apparently only a weak wound-parasite on ginseng and together with strains *H*, *F* and *D*, can be eliminated as a primary causal agent of the disappearing-rot. They may all, however, be important later links in the metabiotic chain.

Experiment 4, 1930, St. Catharines

While Experiment 3 was in progress, a new isolant of *Ramularia*, morphologically quite different from either *M* or *P* appeared in plantings from rotted roots. The pathogenicity of this new isolant, designated *Ramularia R*, was tested on healthy, three-year-old plants growing in experimental plots at St. Catharines. The roots of 10 of these plants were injured and inoculated while 5 similarly injured roots served as checks. The experiment was of 25 days' duration. In the case of the inoculated roots, 100% infection was obtained, while all the checks remained healthy. *Ramularia R* was recovered in 90% of the plantings from the inoculated roots, *Fusarium* spp. in 7%, while representatives of *Penicillium*, *Alternaria*, *Rhizoctonia*, and *Rhizopus* spp., developed from the remaining 3% of the plantings. It was observed in this experiment and in others carried out subsequently that *Ramularia R*, though producing typical rot lesions, does not display the same degree of virulence as forms *M* and *P*.

Experiment 5, 1930-31, Laboratory and Greenhouse

The results obtained in the field experiments were checked by a series of laboratory experiments involving seedlings and older roots. Following surface sterilization by immersion for 30 min. in a solution of formalin, 1½ oz. of commercial formalin to 1 gallon of water, seeds were planted in artificially infested and non-infested soils, steam sterilized in both cases. Healthy roots, surface-sterilized by immersion for six to eight minutes in mercuric chloride (1/1000), followed by rinsing through several changes of sterile water were:

(i) Planted in artificially infested soil, steam sterilized prior to infestation.

(ii) Dipped in or sprayed with a spore suspension of the organism and planted in sterilized soil.

(iii) Injured, inoculated and planted in sterile soil.

The experiments fell naturally into two groups: (a) those in which there was no attempt at careful control of soil moisture and temperature; (b) those which were carried out under conditions of rigid control of these two variables.

Group (a). In this group of experiments the pathogenicity of the three strains of *Ramularia, M, P,* and *R,* and of two strains of *Fusarium, H* and *F,* was tested on roots but not on seedlings. Regardless of how the inoculum was brought into contact with the roots, the three isolants of *Ramularia* never failed to produce infection followed by typical rot symptoms. The two strains of *Fusarium* proved to be weakly parasitic on injured roots, the lesions produced by both strains closely resembling one another but differing from typical disappearing-rot lesions. In general, the results of these experiments closely approximated those obtained in the field.

Group (b). For experiments requiring more rigid control of environmental conditions, three units of modified Wisconsin constant-temperature tanks (8-container capacity each) were used. With this equipment it was possible to investigate, to some extent at least, the relation of soil moisture and soil temperature—probably the most important of several variables—to infection of roots and seedlings.

The soil, a light loam, was autoclaved for 3-hr. periods on each of three successive days. Soil sufficient to fill half the containers was adjusted to 50% of its moisture-holding capacity (hereinafter abbreviated to M.H.C.), while a similar quantity was adjusted to 70% of its M.H.C. Throughout the duration of the experiment—Feb. 19 to April 19, 1931—the containers were weighed daily and sufficient water was added to maintain the required moisture content.

Time and material did not permit the testing of more than one organism. *Ramularia P* was chosen arbitrarily. Preliminary studies had shown that this organism grew on potato-dextrose agar (dextrose, 2½%) at temperatures ranging from 2° to 26–28° C. The top six inches of the soil in half the containers was infested by mixing intimately with it, 60 gm. of oats on which the fungus had been grown for fourteen days.

The roots used in the experiment had been obtained from a garden, which, after a careful survey, appeared to be as free from disease as any in the Waterford district. The required number of roots, following surface sterilization, were planted both in infested and in non-infested soils, at each of the two different moisture contents.

The seeds used in the experiment were all carefully selected, only those being used whose germination was almost assured by the already partial rupturing of the endocarp. They were surface-sterilized and planted, as in the case of roots, in infested and non-infested soils of the two moisture contents. It may be stated here that in seed-treatment experiments involving some thousands of seeds planted in outdoor plots, it had been observed in two successive years that the majority of seedlings emerged from the soil from May 9 to 16, during which period the soil temperature, based on the daily mean at one inch below the surface, averaged 15.4° C., in 1929, and 14.6° C., in 1930.

The tanks were operated at three different ranges of temperatures as follows:

Tank No. 1, 12–14° C., close to optimum for the host.

Tank No. 2, 18–20° C., close to optimum for the parasite.

Tank No. 3, 26–28° C., close to maximum for both host and parasite.

The number of seeds and roots involved in the experiment, together with other data and the results obtained are summarized in Table III.

TABLE III

RELATION OF SOIL MOISTURE AND TEMPERATURE TO ROT INFECTION OF GINSENG ROOTS AND SEEDLINGS

Seedlings

Tank	Temp., °C.	Container	M.H.C. of soil, %	Number of seeds planted		Seedling emergence		Healthy surviving seedlings	
				Sterile soil	Contam. soil	No.	%	No.	%
1	12–14	3	50	42	—	32	76	30	71
		4	50	—	42	8	19	3	7
		10	70	41	—	33	80	32	78
		12	70	—	42	6	14	3	7
2	18–20	2	50	41	—	3	7	3	7
		1	50	—	42	1	2	—	—
		8	70	40	—	4	10	4	10
		11	70	—	42	3	7	—	—
3	26–28	5	50	40	—	—	—	—	—
		6	50	—	42	—	—	—	—
		9	70	43	—	—	—	—	—
		7	70	—	42	—	—	—	—

Roots

Tank	Temp., °C.	Container	M.H.C. of soil, %	Number roots planted		Results		
				Sterile soil	Contam. soil	Healthy roots	Diseased roots	Per cent infection
1	12–14	6	50	4	—	4	—	Trace
		2	50	—	4	—	4	100
		12	70	4	—	4	—	Trace
		8	70	—	4	—	4	100
2	18–20	1	50	4	—	3	1	25
		4	50	—	4	—	4	100
		11	70	4	—	4	—	Trace
		7	70	—	4	—	4	100
3	26–28	5	50	4	—	4	—	—
		3	50	—	4	4	—	—
		10	70	4	—	4	—	—
		9	70	—	4	4	—	—

In Table III, it will be observed that in the 12–14° C., 50% M.H.C., sterile soil, 32 seedlings emerged, 30 of which, representing 71% of the number of seeds planted in container 3, remained healthy throughout the experiment. In the 12–14° C., 70% M.H.C., sterile soil, 33 seedlings emerged, 32 of which, representing 78% of the number of seeds planted in container 10, remained healthy. Even for selected seed, the percentage which germinated was high in both cases, the results thus tending to confirm the observations already made in outdoor experiments in connection with seed germination, that a temperature of about 15° C. is most favorable for the germination of seeds and the development of seedlings. In the 12–14° C., 50% M.H.C., infested soil, eight seedlings emerged, only three of which, representing 7% of the number of seeds planted in container 4, remained healthy. The same small number survived in the 70% M.H.C., infested soil in container 12, thus yielding only six healthy seedlings from a total of 84 seeds planted. It is apparent from the results that *Ramularia P*, even at a point 5° C. below its optimum for vegetative growth, is still extremely pathogenic on ginseng seedlings growing under conditions favorable for their normal development. Since at the two higher ranges of temperature, few or no seedlings survived, the results do not permit of further analysis.

During the period February 19 to April 19, the eight roots planted in the 12–14° C. *sterile* soil of both 50 and 70% M.H.C. (containers 6 and 12), developed normal tops which, at the termination of the experiment, had grown to a height of nine inches. No aboveground parts appeared in any of the other containers. When the roots of these eight plants were examined, it was found that, in general, the main taproots had remained healthy, but, in a number of instances, the tips of some of the laterals were diseased and, in one case, a main taproot had two small lesions on it. In Table III, under "results", these roots are tabulated as healthy, but on account of the slightly diseased condition referred to above, they are also listed under "per cent infection" as showing a trace of infection.

The roots planted in the 12–14° C. *infested* soils of 50 and 70% M.H.C., respectively, were without exception typically rotted, practically the whole of their surfaces being covered with large confluent lesions. The perennial stems showed some evidence of bud development but apparently the disease had progressed with such rapidity as to preclude further growth. *Ramularia P* was re-isolated in 88% of the plantings from these roots.

Of the eight roots planted in the 18–20° C. *sterile* soil of both 50 and 70% M.H.C. (containers 1 and 11), seven remained healthy except for diseased tips of laterals in a number of instances. The other root was found to be badly rotted. The perennial stems of the seven healthy roots showed only slight bud development. In the *infested* soil of the corresponding temperature and moisture series (containers 4 and 7), all eight roots were so badly rotted that it was impossible to remove them intact from the soil. They were in a much more advanced stage of disintegration than those planted in the 12–14° C. infested soil. *Ramularia P* was re-isolated in 84% of the plantings from these roots.

The 16 roots planted in the sterile soil or the infested soil at 26–28° C., remained healthy, all of them showing, however, a general, yellowish discoloration, the direct result, most probably, of the long exposure to the high soil temperature.

The results obtained in the above experiment indicate that the effect due to 6 to 8° differences in soil temperature was much more apparent than that due to a 20% difference in moisture content of the soil. At 12–14° C., in *infested* soil, the disease was not nearly so severe as at 18–20° C., while at 26–28° C. typical rot did not occur. At 12–14° C., the host is near its optimum for growth, while the fungus, on the other hand, is approximately 6° below its optimum. There are, thus, two factors involved whose combined effect tends to modify the severity of the disease. At 18–20° C., the effect of temperature on the host is unfavorable to the extent that the plants cannot proceed beyond slight bud development. On the other hand, the same temperature is most favorable for the growth of the pathogen. There is involved, then, a set of conditions, additive in their effect, which favor optimum disease development with consequent rapid disintegration of the roots. A temperature of 26–28° C. completely inhibits the development of the pathogen, and no disease results. The results of this experiment carried out under carefully controlled conditions in the laboratory confirm those already obtained in the field, indicating that at least one of the pathogens involved is extremely virulent in its attack over a relatively wide range of soil moisture and temperature conditions. There is some indication both from experimental as well as from observational results, that of the two variables, soil moisture and soil temperature, the latter is the more important factor in the incidence of the disease.

Series B. Involving Organisms Isolated from Rusted Roots

In addition to the infection experiments described above, corresponding series were carried out under field and laboratory conditions, using pure cultures of organisms isolated from rusted roots. To date, no definitely positive results have been obtained, but in a laboratory experiment recently completed, in which healthy roots were injured artificially and inoculated with three of five forms of *Ramularia* more recently isolated from rusted roots, certain evidence suggested that these three organisms may bear a causal relation to the rust disease. In this particular experiment, roots inoculated on November 21, 1932, were planted in steam-sterilized soil and kept in a cool cellar. Upon examination on February 21, 1933, several of the inoculated roots showed in the region of injury a depression roofed over by the still intact periderm. The space between the latter and the depressed surface of the root was occupied by a loosely compacted mass of rust-colored tissue closely resembling that found in typical rust lesions and, like the latter, very easily detachable. Free-hand sections from the depth of the concavities showed periderm formation between the superficial diseased tissue and the underlying healthy tissue. In the similarly injured

check plants, the wounds remained clean and healthy and there was no evidence either of the depression or of the masses of diseased tissue which were present in the case of the inoculated roots, but a periderm had formed over the exposed surface of the old injury.

Although the cause of the rust has not been definitely established, it can hardly be questioned that it is a disease entirely distinct from the rot. The circumstantial evidence based on general symptomatology, pathological histology, geographic distribution, association of organisms and results of infection experiments, seems sufficiently convincing to justify the view that the two are different diseases.

Evidence for their occurrence in certain of the ginseng-producing centres of the United States is to be found in Zinssmeister's paper (25), though that author did not realize that he was in all probability dealing with two distinct diseases. He had received specimens of diseased roots from Wisconsin and from New York, all of which he refers to as rusted material. He regarded rusted roots as exhibiting two types of lesions. His description of the first type in which "the injury is merely superficial and is confined to a few layers of cells immediately beneath the epidermis" and where "in many cases the root seems to be able to prevent the further ravages of the disease by cutting off the tissue attacked by the formation of a centripetal cork cambium," is perfect so far as it goes, for the disease known in Ontario as the rust. His description of the second type of lesion which instead of being confined to the sub-epidermal cells, extends to other tissues and finally causes the complete disintegration of the root, is identical with the symptoms of the Ontario disappearing-rot. From the Wisconsin roots which were in an advanced stage of decay and from the New York specimens which showed "the early stages of the disease" two species of *Ramularia* were isolated, both of which, when inoculated into artificially injured but otherwise healthy ginseng roots, were capable of producing "the *deep* type of rot" only. Apparently the superficial type of lesion was never reproduced in any of the infection experiments, which would make it appear that Zinssmeister had succeeded in isolating rot-producing organisms only. His two text-figures of diseased roots, the one showing "a surface view of ginseng roots attacked by *rust*", the other, "*rusted* roots in longitudinal section showing the *deep* type of lesion" are good illustrations of roots affected with the disappearing-rot.

It would appear that in the diseased specimens Zinssmeister obtained from the two widely separated sources, both the rust and the rot were present, either on different or, possibly, in some cases, on the same roots. This would not be inconsistent with what has been observed in Ontario, for the two diseases have been found encroaching upon roots in the same beds in certain gardens. There seems little doubt that the serious losses which for years have been incurred in many of the ginseng-growing centres of the United States and which have been attributed to the rust disease, are due, as in Ontario, not to one but to two distinct diseases.

The Pathogens

Literature Review with Reference to Isolation of Ramularia from Soil and from Underground Parts of Plants

Since Unger in 1833 established the genus *Ramularia*, it has been steadily augmented by the addition of new members until Saccardo (8, volumes 1 to 25 inclusive), records approximately 450 species, which with few exceptions have been found in association with the aboveground parts of plants. There are comparatively few references in the literature to members of the genus having been isolated either directly from the soil or from the underground parts of plants.

According to Wollenweber (24) in 1913, who gave not only the complete taxonomic history of the genus but also a revised generic diagnosis, five species of *Ramularia*, *R. candida*, *R. magnusiana*, *R. eudidyma*, *R. macrospora* and *R. olida*, were up to that time known to have been isolated from different underground parts of plants in widely separated districts. Only one of these, *R. olida*, had been found exclusively in association with subterranean parts of plants, the other four having been isolated, in addition, from leaves, stems and fruits. As the result of cross-inoculation experiments involving these different species, Wollenweber states that, "Ramularien, die als Blattparasiten bekannt sind, rufen ausserdem Knollen—und Fruchtfäule hervor und finden sich ausser auf Blättern auf unterirdischen Organen und ferner im Erdboden und auf Mist vor"; also that "Die Gattung Ramularia enhält eine Reihe ubiquitischer Wundparasiten". Sherbakoff (9), described and named a new species, *R. solani*, which was isolated from diseased tissue of a potato tuber affected with a superficial dry rot. Zinssmeister's work (25), demonstrating that *R. panacicola* and *R. destructans* n. spp. cause the disease of the roots of American ginseng popularly known as rust, is probably the first instance in which it has been shown that members of the genus are parasitic on the roots of any host plant. During investigations of yellow-leaf disease of *Phormium tenax*, Waters and Atkinson (15) isolated *R. phormii* from the roots of affected plants, in the tissues of which it was fructifying. Williams *et al.* (22) record a *Ramularia* sp. in connection with a root rot of *Statice latifolia*. Berkeley (1) isolated *Ramularia* spp. from rusted ginseng roots and in a single instance a *Ramularia* sp. was obtained from rot-infested soil. In 1927, Gilman and Abbott (4) published a summary of the fungi of the soil, according to which *R. eudidyma* Wollenw., and *R. macrospora* Fred., had been isolated from soil in Europe, while *R. magnusiana* had been obtained from soil in Texas. Walker (14) reports having obtained isolants of *Ramularia* during several seasons from diseased strawberry roots. Nobles (6) obtained from virgin soil in the vicinity of Toronto, Ontario, five isolants of *Ramularia* which were identified only as far as the genus.

Taxonomy

All isolants of *Ramularia* encountered in the present investigation were identified as such on a basis of Wollenweber's diagnosis of the genus (24,

p. 220). Specific identification was attempted only in the case of the three forms *M*, *P*, and *R* which had been shown definitely to be causal agents of the disappearing-rot. The three forms were studied critically in pure culture and their morphological and cultural characters were carefully compared with those of the definitely identified species recorded in the literature as having been isolated either from the soil or from the underground parts of plants. In studies of spore septation, and also for photographic purposes, the spores were mounted directly in a weak, aqueous solution of ruthenium red, following the suggestion of Brown and Horne (3) who employed this technique with success in their studies in the genus *Fusarium*. Spore samples for measurement were taken 1 cm. from the centre of 14-day-old petri dish cultures, grown at room temperature on potato-dextrose agar (2½% dextrose), pH 5.5, careful studies of the three forms at different stages of their growth having shown that at about 14 days they reached a stage of maturity when the conidia were of most uniform and typical shape and size. Photomicrographs were obtained using the Vernon (11) set-up which was found very well adapted to studies of this kind.

Ramularia P was found to correspond so closely with Zinssmeister's (25) *R. panacicola* as to leave little doubt that the two pathogens are identical. Two thousand four hundred conidia of *Ramularia P* (Plate II, Fig. 1) were found to measure 2.1–7.1 × 5.2–33.9 μ. Zinssmeister's measurements for 4,000 conidia are given as 2.5–7.2 × 5.5–34.2 μ. With regard to septation of conidia there is the close agreement shown in Table IV. Close uniformity in other morphological characteristics, including color of mycelium, size and color of chlamydospores and thickness and consistency of stroma in agar cultures, further confirm the identity of the two organisms.

TABLE IV

COMPARISON OF ZINSSMEISTER'S *R. panacicola* WITH *Ramularia P*

Organism	Number of conidia examined	Septation		
		0	1	2
Ramularia P	3000	69.6%	30.3%	.06%
R. panacicola	8000	69.0%	31.0%	—

Ramularia M cannot be identified with any of the species recorded in the literature as having been isolated either from soil or from the subterranean parts of plants. Two thousand conidia were found to measure 2.1–5.1 × 4.6–50.8 μ, 85% ranging in length from 5.0–19.0 μ. Whereas the larger conidia of *R. panacicola* (Plate II, Fig. 1) tend to be slightly pyriform, those of *Ramularia M* (Plate II, Fig. 2) are, in general, longer and more narrowly cylindrical. They are abstricted successively at the apices of simple, sparingly branched, or verticillately branched conidiophores, smaller spores being cut off first, followed by larger ones from the same conidiophores (Plate II, Fig. 6). Of 5,770 conidia examined as to septation, 83.6% were found to be 0-septate, the remaining 16.4% being 1-septate. The chlamydo-

spores of the two organisms are, in general, indistinguishable from one another and show wide variation in shape and size. Plate II, Fig. 5, shows an extreme in variation from the more constantly occurring type shown in Plate II, Fig. 4. Young cultures of the two organisms on potato-dextrose

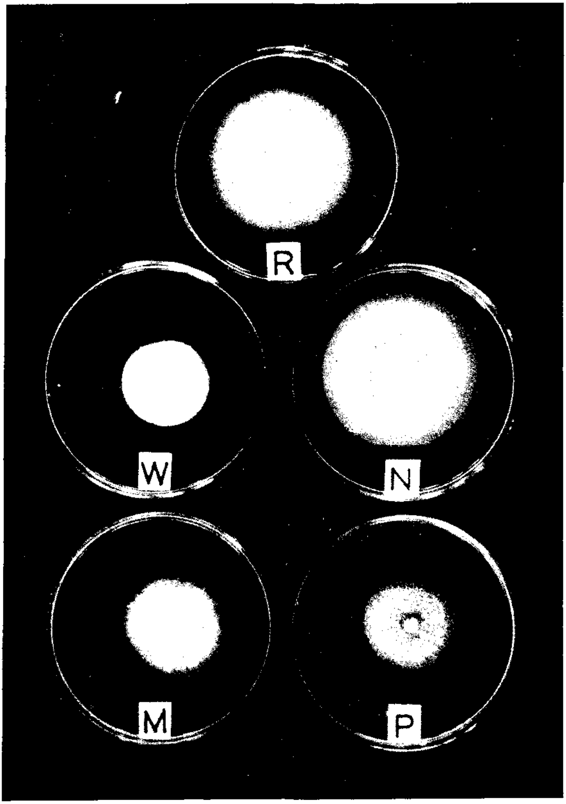

FIG. 2. *Ten-day-old cultures of R. robusta (R), R. panacicola (P), R. mors-panacis (M) from ginseng, Ramularia W (W) from strawberry and Ramularia N (N) from soil, incubated at 16° C., on potato-dextrose agar.*

agar (2½% dextrose) closely resemble one another, but whereas the periphery of the colony of *R. panacicola* is always regular and sharply defined (Text-fig. 2, P) that of the colony of *Ramularia M* tends to be more or less irregular and indefinite, the hyphae of the latter organism merging almost imperceptibly into the substratum (Text-fig. 2, M). Young cultures of the two organisms are more readily distinguishable when grown on a starch-containing medium prepared according to the direction of Paul (7). *Ramularia M* like *R. panacicola* forms a thick leathery stroma on various agar substrata. In addition to the greater range in size of conidia, the higher percentage of non-septate spores and the less sharply defined edge of colony in agar culture, there are also certain other morphological and physiological characters in which *Ramularia M* differs from *R. panacicola*, sufficiently, it is believed, to be regarded as a distinct, new species for which the binomial *Ramularia mors-panacis* is proposed and which is described as follows:

Ramularia Mors-panacis sp. nov.

Technical Description

Conidia, hyaline; first-formed conidia smaller, ovoid to short cylindrical with ellipsoidal ends; later-formed conidia larger, fusiform or long narrowly cylindrical (Plate II, Fig. 6), infrequently papillate, not constricted at the septum; 0- to 1-septate, 84% of 5770 conidia, 0-septate, not producing chlamydospores endogenously; anastomosis of germ tubes rare; 2.1–5.1 × 4.6–50.8 μ, 85% of 2000 conidia ranging in length from 5.0 to 19.0 μ; conidia abstricted successively at the apices of simple, sparingly branched, or verticillately branched, septate conidiophores which may be scattered on the aerial mycelium or in sporodochia arising from the stroma; sterigmata more or less tapering, single or in groups up to four; aerial mycelium (on potato-dextrose agar (2½% dextrose), pH 5.5) abundant, septate, branched, at first white, loosely compacted, later showing transition through old-gold (R)* to Dresden-brown (R), two to several hyphal strands commonly aggregating to form coremium-like wefts; periphery of colony often irregular and indefinite; stroma thick, leathery, darker than claret-brown (R); chlamydospores abundant, intercalary, in chains or pseudo-sclerotial masses, darker than chestnut (R), irregular to spherical, the latter 7.8–23.4 μ (300 measured) in diameter; ascigerous stage not known.

Habitat in living roots of *Panax quinquefolium* L., Ontario, Canada.

*Ramularia Mors-panacis, sp. nov.***

Conidiis hyalinis, aliis primum formatis, minoribus, ovoideis vel breviter cylindraceis; deinde aliis majoribus, fusoideis vel longis et anguste cylindraceis, raro papillatis, in septo non constrictis; omnibus 0–1 septatis sed 84 per centum 0-septatis (5770 conidiis probatis), chlamydosporis non intra natis praeditis, 2.1–5.1 × 4.6–50.8 μ, quorum duorum milium 1700 conidia 5.0 usque ad 19.0 μ sunt longa, ab apicibus simplicium et raro vel longitudi-

**The symbol (R) is used here and subsequently to denote that the color nomenclature is that of Ridgway. Ridgway, Robert. Color standards and color nomenclature. 1912.*

****Latin diagnoses in this paper by Miss M. H. Thomson, 586 Spadina Avenue, Toront*

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naliter ramificatarum conidiophorarum quae sparsa vel in sporodochiis posita sunt aliis post alios abruptis; sterigmatibus singulatim vel usque ad quattuor gragatim positis; mycelio aerio, in agar cultura innato, copioso, septato, ramoso, primo albo, deinde old-gold (R) vel dresden-brown (R), hyphis duabus vel pluribus fere innexis atque texturas formantibus; coloniis perimetro saepe irregulari indefinitaque praeditis; stromatibus crassis, lentis, obscurioribus quam claret-brown (R), chlamydosporis copiosis, intercalaribus, concatenatis vel in falsis sclerotiis positis, atrioribus quam chestnut (R), aliis irregularibus, aliis globosis, his 7.8–23.4 μ diam., evolutione sexuali ignota.

Hab., radicibus vivis *Panacis quinquefolii* L., Ontario, Canada.

Ramularia R like *R. mors-panacis* cannot be identified with any of the species recorded as having been isolated from the soil or from the underground parts of plants. This organism is more conspicuously different from either *R. panacicola* or *R. mors-panacis* than are the latter from each other. The conidia (Plate II, Fig. 3) are not only much larger but are also more variable in shape and may have as many as eight septa. Five thousand six hundred and twenty conidia were found to range in size from 1.4–9.1 × 3.1–122.0 μ. Approximately 98% of the conidia are less than 65.0 μ in length and do not possess more than three septa but included in the remaining 2% are "giant" conidia, the largest observed measuring 122.0 μ in length. In agar cultures smaller conidia are abstracted successively at the tips of simple to sparingly branched conidiophores (Plate II, Fig. 8) arising from the aerial mycelium which, as in the case of both *R. panacicola* an *R. mors-panacis* consists either of individual hyphae or of strands of the latter aggregated into coremium-like wefts. The smaller conidia often adhere to the tips of the conidiophores in small gelatinous heads. Larger conidia occur most profusely as pionnotes on the surface of various agar substrata. On the latter, *Ramularia R* forms a thin stroma and, in old or desiccated cultures, chlamydospores, brown in color and mostly intercalary in position, are produced but much less abundantly than in the case of the other two ginseng pathogens. *Ramularia R* grows much more rapidly at room temperature than either *R. panacicola* or *R. mors-panacis*. *Ramularia R*, in addition to its conspicuously larger conidia, its thinner stroma and relative paucity of chlamydospores together with its faster growth rate, exhibits other morphological, physiological and pathological characteristics which mark it as so distinctly different from *R. panacicola* and *R. mors-panacis* as to warrant regarding it as a distinct, new species for which the binomial *Ramularia robusta* is proposed and which is described as follows.

Ramularia Robusta sp. nov.

Technical Description

Conidia hyaline; smaller conidia ovoid to broadly cylindrical, abstricted at the apices of simple to sparingly-branched conidiophores arising from the mycelium, often adhering to the conidiophores in small gelatinous heads; larger conidia broadly cylindrical, clavate, mostly slightly curved, infrequently papillate, not forming chlamydospores endogenously and not constricted at

the septa but giant conidia often variously curved and distorted, occurring most profusely as pionnotes covering surface of stroma; 0–8-septate; 1.4–9.1 × 3.1–122.0 μ (5620 conidia examined as to size and septation); 33%, 1-septate, 4.0–8.2 × 10.5–37.4 μ, average 5.9 × 21.6 μ; 4.8%, 2-septate, 5.4–8.4 × 24.5–38.6 μ, average 6.9 × 30.8 μ; 5.2% 3-septate, 5.6–9.1 × 27.6–65.3, average 8.2 × 42.5 μ; aerial mycelium (on potato-dextrose agar (2½% dextrose), pH 5.5) septate, branched, at first white, soon becoming buffy-brown (R), sparse, later merging with the substratum and giving the surface a mealy appearance; anastomosis of hyphae frequent and two to several strands aggregating to form coremium-like wefts; stroma, thin, seal-brown (R); chlamydospores in old or desiccated cultures mostly intercalary, in chains or pseudo-sclerotial masses, buffy-brown (R), mostly irregular, at times spherical, the latter up to 14.5 μ in diameter; ascigerous stage not known.

Habitat in living roots of *Panax quinquefolium* L., Ontario, Canada.

Ramularia Robusta sp. nov.

Conidiis hyalinis, aliis minoribus ovatis vel late cylindraceis, ab apicibus simplicium vel parce ramosarum et in mycelio sparsarum conidiophorarum abruptis, saepe in eis parvis gelatinosis globis adhaerentibus; aliis majoribus, creberrime quam pionnatibus, late cylindraceis, clavatis, fere paulo flexis, infrequenter papillatis, chlamydosporas non intra formantibus neque ad septa constrictis sed maximis saepe varie curvatis et detortis; omnibus 0–8-septatis, 55 per centum 0-septatis, 33 per centum 1-septatis, 5 per centum 2-septatis, 5 per centum 3-septatis; 1.4–9.1 × 3.1–122.0 μ, quorum 5620 omnium 5508 conidia non longiora sunt quam 65.0 μ; mycelio aerio, in agar cultura alito, septato, ramoso, primo albo, mox buffy-brown (R), sparso, demum substrato conjuncto hoc et superficies granulosa videatur efficiente; anastomosibus inter hyphas frequentibus, et duabus vel pluribus hyphis fere innexis atque texturas formantibus; coloniis perimetro regulari et definita instructis; stromatibus tenuibus, seal-brown (R); chlamydosporis in veteribus vel desiccatis culturis crescentibus, plerumque intercalaribus, concatenatis vel in falsis sclerotiis positis, buffy-brown (R), multis irregularibus, sed paucis globosis, his usque ad 14.5 μ diam.; eis que ad generationem pertinent ignotis.

Hab., in radicibus *Panacis quinquefolii* L., Ontario, Canada.

To date the three species have remained remarkably uniform in culture. After a long series of transfers the conidia and chlamydospores show no diminution in quantity or size. A sexual stage has never been observed and apparently cannot be induced either by subjecting the three organisms to varying environmental conditions or by growing them together in culture.

Spore Germination

Conidia of the three species will readily germinate in two hours in tap water at 20° C. In 24 hours at room temperature, chlamydospores will germinate (Plate II, Fig. 7) as readily as conidia in drops of tap water. Non-septate spores show the same facility for germination as those which possess septa.

Relation of Temperature to Growth in Pure Culture

The three species from ginseng, an isolant from diseased strawberry roots, supplied by A. R. Walker (14), and one of Miss Nobles' (6) isolants from virgin soil were grown under carefully controlled environmental conditions, in petri dishes on potato-dextrose agar ($2\frac{1}{2}$% dextrose), at 2° C. intervals over a temperature range of 0–30° C. At each temperature interval, each of the five isolants was grown in triplicate, thus totalling fifteen plates per series. Data were recorded for two complete and independent series involving approximately 450 plate cultures. The cultures were incubated for ten days at each temperature interval, at the end of which time maximum radial growth was determined on the basis of diameter of colony.

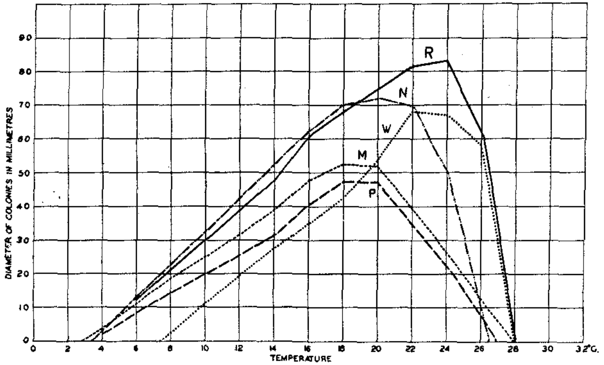

FIG. 3. *Curves showing relative growth of R. panacicola (P), R. mors-panacis (M), R. robusta (R) from ginseng, Ramularia W (W) from strawberry, and Ramularia N (N) from soil, as indicated by diameter of colonies of the five organisms, after incubation at 2-degree intervals for 10 days on potato dextrose agar.*

The three species from ginseng have practically the same growth range, from about 3 to 28° C. (Text-fig. 3). The optimum for *R. mors-panacis* and *R. panacicola* occurs about 18–20° C., while that of *R. robusta* is nearer 24° C. The latter, throughout its range, exhibits more abundant growth than the other isolants, with the exception of *N*, the isolant from soil. *R. mors-panacis* and *R. panacicola*, which closely resemble one another in their pathogenicity, exhibit the same general temperature requirements upon artificial media, their growth curves showing a close parallelism, with *R. panacicola* lagging slightly behind *R. mors-panacis*. The isolant from strawberry roots, *W*, with its minimum for growth at about 7° C. and its optimum from 22 to 24° C., exhibits higher temperature requirements than *R. mors-panacis* and *R. panacicola* from ginseng.

Longevity in the Soil

At various times during the course of the present investigations attempts were made to isolate the pathogens from soil samples taken from plots where the disease was definitely known to have been especially severe. Soil dilutions varying in concentration from strong to weak (1 : 500 to 1 : 1,000,000) were mixed with, or applied to, the surface of various media which are reported as having been used with success by other workers investigating fungi of the soil (4, 6, 13, 16). Plates were incubated over a range of temperatures which included the optima for vegetative growth of the three rot-producing organisms. To date, neither the latter nor any other representative of the genus *Ramularia* ever appeared in any of over 400 poured plates. It remains impossible, therefore, to state definitely how long or in what form the pathogens may persist in the soil but evidence from field observations indicates that they remain viable for considerable lengths of time. The numerous failures to produce second crops of ginseng in soil that has once become infested and the increasing number of gardens abandoned because of ginseng-sick soil both point to the ability of the pathogens to live over and accumulate in the soil.

Cross Inoculation Experiments

The occurrence of individual rotted roots variously scattered throughout an otherwise healthy stand of ginseng is difficult to account for. The only possible explanation in certain cases would seem to be the presence of a pathogen residual in the soil. Miss Nobles' (6) discovery of representatives of the genus *Ramularia* residual in virgin soil in Ontario seemed particularly significant. If it could be shown that a *Ramularia* residual in the soil was pathogenic on ginseng, this might explain the occurrence of disease in new stands grown from treated seed. Especially interesting in this connection is the following excerpt from Werkenthin (16) who, in 1916, investigated the fungous flora of Texas soils, "Of special interest in the study of soil fungi is the fact that the virgin soil contained fungi which are known to be parasitic to cultivated plants, *e.g.*, *F. solani*, *F. oxysporium* and *F. radicicola*. It also should be noted that these fungi were isolated several times from the same plot during a period of over five months, which fact should show clearly that these fungi are true inhabitants of the soil". Of interest, too, in connection with the present investigations was Walker's (14) assigning the cause of strawberry root rot in the Niagara Peninsula to a *Ramularia* sp. In the Waterford district, strawberry root rot is becoming as serious a problem as ginseng root rot and in many instances ginseng and strawberries are grown in close proximity to one another. In view of the possibilities suggested by the above findings, the following series of pathogenicity tests were carried out.

Series 1. Pathogenicity of Isolants of *Ramularia* from Strawberry and from Soil, on Ginseng

Healthy, two-year-old roots, were immersed for 8–10 min. in 1 : 1000 bichloride of mercury, then rinsed through three changes of sterile water, and artificially injured by making an incision ¼ to ⅜ in. long, using a flamed scalpel. Inoculum from 14-day-old tube cultures of forms *W* (strawberry), *N* (soil), and the three species from ginseng was inserted into the incision. Check roots were similarly injured but not inoculated. The roots were planted in sterile soil in pots which were left in a cool cellar for 36 days, sterile water being added as required. Sixty roots were involved in the experiment, 10 serving as checks and 10 being inoculated with each of the five isolants of *Ramularia*.

On all 10 roots inoculated with *Ramularia W*, small necrotic areas developed in the immediate region of the injury (Plate III, Fig. 1, W). The organism was recovered in 90% of the plantings from necrotic tissue.

On all 10 roots inoculated with *Ramularia N*, definite lesions developed in the region of injury (Plate III, Fig. 1, N). The lesions, however, were not typical of those of the disappearing-rot (Plate III, Fig. 1, M, P and R), being more sharply delimited both externally and internally from healthy tissue and, in color, a light, yellowish brown rather than dark, reddish brown. The organism was re-isolated from 87% of the plantings from diseased tissue.

The check roots all remained healthy (Plate III, Fig. 1, C). Results obtained earlier with the isolants from ginseng were confirmed.

The results recorded above, which were confirmed in subsequent tests, demonstrate that the five isolants of *Ramularia* possess different infection capabilities where ginseng is concerned. On this host, the isolant from strawberry is only slightly pathogenic, the soil organism exhibits a somewhat greater degree of virulence, and, as has been demonstrated consistently, the other three isolants are aggressive parasites, *R. robusta*, however, not displaying the same degree of virulence as the other two species (Plate III, Fig, 1, M, P and R).

Series 2. Pathogenicity of Isolants of *Ramularia* from Ginseng and from Soil, on Strawberries

On July 22, 1931, strawberry plants, variety Glen Mary, grown for 42 days in the greenhouse from runners trained over sterile soil in 5-in. pots were transferred to 9-in. pots containing soil which had first been sterilized and then infested with *R. panacicola* and *R. mors-panacis* (ginseng), and *Ramularia N* (soil), growing on sterilized oats. Thirty-two plants were involved in the experiment, three lots of eight each being transferred to soil infested with *R. panacicola*, *R. mors-panacis* and *Ramularia N*, respectively, while the remaining eight plants in non-infested soil served as checks. The 9-in. pots were transferred to outdoor plots where they were sunk until their tops were about two inches above the level of the surrounding soil. Through-

out the remainder of the 1931 growing season no difference could be noted between plants growing in infested and non-infested soil. Further observations were not possible until May 25, 1932.

On that date, a striking difference was noted between the plants grown in the soil to which *Ramularia N* had been added and the remainder of the plants. Of the former, four had almost completely disappeared except for a few shrivelled remains of tops and roots, two were dying and, when removed from the soil, were found to have a very limited and badly diseased root system, and the remaining two plants, though still living, were "dwarf plants" with correspondingly limited root systems. From brownish, discolored areas on the roots of the stunted and dying plants, 60 tissue plantings were made and *Ramularia N* was recovered from 86% of the plantings.

One of the plants grown in the soil infested with *R. mors-panacis* was dead, apparently having died, however, before growth was resumed in the spring. The remaining seven plants of the series had well developed root systems which appeared to be perfectly healthy except for the occurrence on a few laterals of scattered lesions from which *R. mors-panacis* was recovered in a few instances.

In the case of plants grown in soil infested with *R. panacicola* the results obtained were almost identical with those described above for *R. mors-panacis* except that all of the plants of the series had remained healthy. From a few brownish, discolored areas on a number of the laterals, *R. panacicola* was recovered in pure culture.

One of the eight check plants was dead, apparently having died during the winter as there was no indication of current-year growth. The remaining seven plants had healthy, well developed tops and root systems.

From the results obtained in the above experiment and from the preceding one, it would appear that the presumably saprophytic isolant from the soil, *Ramularia N*, can, under certain conditions, at least, become parasitic on the roots of certain living plants. On strawberry roots the organism is much more aggressive in its attack than on ginseng roots. The results of this experiment also suggest a marked degree of specificity on the part of two of the species isolated from ginseng. These species which are such aggressive parasites on ginseng cannot attack healthy roots of the variety of strawberry involved in the experiment. This fact was confirmed later in greenhouse experiments.

SERIES 3. PATHOGENICITY OF ISOLANTS OF *Ramularia* FROM GINSENG, FROM STRAWBERRY AND FROM SOIL, ON CARROTS

Forty-eight healthy carrots were surface-sterilized for 8 to 10 min. in 1 : 1000 bichloride of mercury and then rinsed through three changes of sterile water. They were then apportioned at random into six groups of eight each. Four from each group were artificially injured by making an incision about ½ in. long, with a flamed scalpel. These narrow incisions were packed with inoculum obtained from 14-day-old cultures of the five isolants of *Ramularia*.

Injured checks were treated in identically the same manner using sterile agar The four remaining carrots of each group were dipped in spore suspension of each organism. All were planted June 8, 1932, in sterilized soil in pot which were transferred to the outdoor plots where they were left until July 11 sterile water being added as required.

Every carrot produced an abundant growth of green, healthy tops and a new root system, a response in marked contrast to the passive behavior o ginseng roots subjected to similar treatment. Towards the end of the experiment the tops of the plants which had been injured and inoculated with *R. robusta* showed signs of wilting. On July 11, when the roots were examined it was found that of the roots which had been artificially injured only those which had been inoculated with the species of *Ramularia* isolated from ginseng were diseased (Plate III, Fig. 2, M, P and R). *R. robusta*, which on ginseng is always less virulent in its attack than *R. panacicola* or *R. mors-panacis*, displays the greatest degree of virulence on carrots, causing a much greater disintegration of root tissues in 33 days than either of the other species. All three species were recovered in a high percentage of cases from the respective roots into which they had been inoculated. All the roots injured and inoculated with isolants *N* and *W* remained healthy, as did the checks (Plate III, Fig. 2, N, W and C).

In the case of the carrots inoculated by immersion in spore suspensions of the five organisms, the results closely paralleled those described above. Only those roots which had been dipped in spore suspensions of the three isolants from ginseng became diseased, but in no instance were they as severely attacked as when injured and inoculated. Again *R. robusta* was the most virulent pathogen. All the carrots inoculated with isolants *N* and *W* remained healthy, as did the checks.

The results described above, which were confirmed in other experiments, suggest further the degree of specificity exhibited by representatives of the genus *Ramularia* obtained from different sources. The isolant from strawberry, which in a previous experiment was shown to be slightly pathogenic on the roots of ginseng, does not attack carrots even when an infection court is provided. The same is true for *Ramularia N*, the soil isolant, which, however, shows marked aggressiveness in its attack on the roots of strawberry and is also pathogenic to a degree on ginseng. *R. mors-panacis*, *R. panacicola* and *R. robusta* show marked aggressiveness in their attack both on ginseng and on carrots, but the two first-mentioned species when tested on strawberries did not attack that host.

Seed Treatment Experiments

Believing that the dissemination of disease is associated with the seed and that remedial measures are to be found in the disinfection of the seed, many of the growers in Ontario have adopted a method of seed treatment which involves immersion in a solution of formaldehyde. The effect of such treatment on the germination of the seeds, especially when the seed coat is already

cracked open, whether the diseases really are seed-borne and, if so, the effectiveness of the treatment in combating them, remained to be investigated.

In September 1930, 1200 seeds with the seed-coats cracked open were selected from a quantity of seeds which, having been picked the previous autumn, had passed through twelve of the eighteen months of their natural period of dormancy and should, therefore, germinate the following spring. They were apportioned at random into four lots of 300 each. Lot A was suspended in sterile water for one hour; lot B was immersed in a solution of formaldehyde (2 oz. per gal.) for one hour; lot C was soaked for one hour in a heavy spore suspension of *R. panacicola*, in which both conidia and chlamydospores were abundant; lot D, after soaking in the same spore suspension for one hour, was immersed in the formaldehyde solution (2 oz. per gal.) for one hour. The strength of solution and the period of immersion were chosen as being most closely in accordance with the treatments most generally in use in the Waterford district. Without being allowed to dry, the seeds were planted, Sept. 28, 1930, in an outdoor plot, the soil of which was covered with a mulch of leaves.

The numbers of seeds involved, the treatments to which they were subjected and the resultant percentage germinations as evidenced by seedling emergence May 4 to May 23, 1931, are summarized in Table V.

TABLE V

EFFECT OF FORMALDEHYDE TREATMENT ON GERMINATION OF GINSENG SEEDS AND ON VIABILITY OF TYPICAL SPORE FORMS OF *R. panacicola*

No. seeds planted	Treatment	Germination %
300	One hour in sterile water	84.6
300	One hour in 2-ounces-to-the-gallon formaldehyde solution	88.4
300	One hour in spore suspension of *R. panacicola*	8.7
300	One hour in spore suspension of *R. panacicola* followed by one hour in 2-ounces-to-the-gallon formaldehyde solution	77.4

From the results of the experiment as recorded in Table V, it would appear that soaking seeds, even when cracked open, for one hour, in a solution of formaldehyde (2 oz. per gal.), rather than having an adverse effect, tends to stimulate their germination, also, that such treatment is most effective in destroying the viability of conidia and chlamydospores of *R. panacicola* present on the surface of the seeds.

An experiment similar to the one described above but involving about 3600 seeds was performed in the fall of 1931 and the results obtained in the 1930-31 experiment were closely confirmed. Again it was noted that the formalin-treated seeds showed about 4% increase in germination over those treated with sterile water alone.

Having adduced some evidence that the formaldehyde treatment, as generally employed, would be effective in controlling the rot if it were a case of th typical spore forms of the pathogen being carried mechanically on the surfac of the seed, there still remained the possibility of failure of such treatmen to control the disease if the parasite were localized within the seed coat o in the reproductive organs of the seed. Mature "berries" were collected fron plants whose roots were typically rotted. Material including the fleshy meso carp and the endocarp with its contents, was fixed, sectioned, stained an examined microscopically. No evidence of the presence of a pathogen in an of the tissues was found in the material examined. Other "berries" wer surface sterilized and tissue plantings from the mesocarp were made o potato-dextrose agar. In other cases, following surface sterilization of th epicarp and the removal of the mesocarp aseptically, the endocarp with it contents was transferred, in whole or in part, to the same nutrient medium In many series of such plantings, the rot-producing organisms never appeare in culture.

In addition to the above studies, many sections from various regions of th stems of plants with diseased roots have been examined microscopically, an plantings have been made to various nutrient media. In not a single instanc has it been possible to demonstrate the presence of the pathogens in any par of the stem above the ground level. All the evidence, both from laborator experiments and from observations in the field, points to the fact that th rust and the rot are not systemic diseases but are confined to the parts of th plant that remain underground.

While it is believed that neither the rust nor the rot is transmitted throug the seed and that ordinarily the pathogens are not present on the surface o the seeds, it is not considered impossible that at some stage in their handlin they may become contaminated. Not only as a safeguard against the latte contingency but also as a possible important factor in the control of anothe important disease of ginseng, the leaf and stem blight caused by *Alternari panax* Whetzel, the spores of which fungus have been found in abundanc on the surface of a large number of seeds examined in the present investigations, it is recommended that all seeds, preferably just prior to planting be immersed for a period of one hour in a solution of formaldehyde (2 oz per gal.), care being taken to plant the seeds before they become dry. Suc treatment has been found to increase germination to a slight but appreciabl extent (about 4%).

Control

With either or both of the diseases already firmly established in the principa ginseng-growing districts of Ontario, exclusionary or eradicatory measures fo their control offer very limited possibilities. Brann (2) reported that result obtained in Wisconsin following steam sterilization of soil by the inverted pan method indicated that the process was at least partially effective i controlling the brown root rot, or rust, of ginseng. Under existing economi

conditions sterilization of soil by heat is as impracticable to the majority of the ginseng growers in Ontario as is soil disinfection by the application of chemicals, except where relatively small areas are involved. With a view to obtaining information as to the possibility of effectually preventing the spread or occurrence of the diseases by the application of disinfectants, a series of experiments involving the use of formaldehyde, Bordeaux mixture, copper sulphate solution, bichloride of mercury, sulphur and paradichlorobenzene, on small areas of either artificially or naturally infested soils have been carried out in laboratory experimental plots and in the field at Waterford. The results in general have been of such a contradictory nature that it remains impossible to recommend with certainty the use of any of the above-mentioned chemicals for the effectual control of either the rust or the rot when they make their appearance in a stand of ginseng.

In the hope of securing resistant varieties certain of the growers at Waterford have visited gardens in the United States and from those which appeared to be free from disease have secured seeds and young roots for transplanting. The general experience has been that imported stock has proved to be as susceptible to rust and rot as native stock. Nor does the possibility of securing disease-resistant plants by selection seem to offer much hope as a factor in control. During the past four years it has been observed that when the rot spreads from a centre of infection almost invariably every plant succumbs. Even if certain plants did survive and seeds were obtained from them, the task of developing a disease-resistant strain would be a particularly long and arduous one. Ginseng seeds do not germinate for 18 months from the time they are picked and plants do not produce their first crop of seeds until the third growing season.

Discussion

In the course of the present investigations ten representatives of the genus *Ramularia* have been found more or less consistently in association with a diseased condition of ginseng roots. Five of them were isolated from typically rusted roots and five from characteristically rotted roots. Three of the five isolants from the latter source, which have been shown to bear a primary causal relation to the rot disease, have been studied intensively in an attempt to identify them with the relatively few species of the genus reported as having been isolated either directly from the soil or from the underground parts of plants. No effort was made, however, to establish their identity with any of the large number of species (approximately 450) which have been found in association with the aboveground parts of plants. One of the pathogens, referred to in the body of the paper as *Ramularia P*, was identified as *Ramularia panacicola* Zins., but the other two, designated as *Ramularia M* and *Ramularia R*, could not be identified. Pathologically, *Ramularia M* closely resembles *R. panacicola* but differs from the latter in certain morphological, cultural and physiological characteristics. *Ramularia R* differs distinctly from the other two pathogens, morphologically, pathologically and

physiologically. The evidence which has been presented is regarded as sufficient to warrant the description of *Ramularia M* and *Ramularia R* as two new species for which are proposed the binomials *R. mors-panacis* and *R. robusta*.

The present investigations have also given some indications of the parasitic capabilities of at least five different representatives of the genus. Cross-inoculation experiments involving unrelated hosts and isolants of the fungus obtained from entirely different sources have indicated variations in pathogenicity among the isolants as well as specificity in host relationships. An isolant from virgin soil (*Ramularia N*) has been shown to be an aggressive parasite on the roots of strawberry. The same organism is pathogenic to a degree on the roots of ginseng but possesses no capacity for attacking the roots of carrots even when an artificial infection court is provided. Three isolants from ginseng, *R. panacicola* Zins., *R. mors-panacis* n. sp., and *R. robusta* n. sp., all aggressive parasites on that host, exhibit marked differences in their virulence, the two first-named species being capable of bringing about much more rapid disintegration of the host than *R. robusta*. The latter species, however, is much more virulent in its attack on carrots than the other two ginseng pathogens. An isolant from strawberry (*Ramularia W*) is slightly pathogenic on the roots of ginseng but cannot attack the roots of carrot even when the internal tissues have been exposed by injury. It may be mentioned here that, incidental to the present studies, ten additional representatives of the genus *Ramularia*, different from each other and from the ten isolants obtained from ginseng, have been isolated from definite and characteristic lesions occurring upon otherwise healthy, functional tissue of (i) the rootlets of apple, (ii) the suckers of raspberry, and (iii) the finer lateral roots of both strawberry and *Agropyron repens*. An additional isolant has also been obtained from the older roots of grape, showing discoloration of vascular tissue. In the light of the above it would appear that the genus *Ramularia* must be regarded as an important member of the group of facultative parasites associated with root troubles of plants.

An interesting feature of the present work is the demonstration that representatives of the genus *Ramularia* occur more abundantly in association with the underground parts of plants than the literature to date would indicate. If a concerted effort were made to examine the subterranean parts of a still wider range of possible host plants, it is believed that this fungus would be shown to occur as ubiquitously in a soil environment as it does on aerial parts of plants. Why it has not been reported more frequently by other workers investigating root troubles of plants is difficult to understand. One reason may be that it has been overlooked. The members of the genus isolated in the present work grow, in general, much more slowly at room temperature than the representatives of several of the genera so frequently encountered in studies of this kind, including *Fusarium, Penicillium* and members of the *Mucoraceae*. Unless mixed cultures developing from tissue plantings are examined most carefully, the presence of *Ramularia* can easily

be overlooked. Tube cultures in particular, because of the early appearance of fungi regarded as contaminants, are no doubt discarded in many instances before the presence of *Ramularia* can be detected. Another reason why the organism has not been reported more frequently may be that it has been mistaken for other fungi. Certain members of the genus are, morphologically, quite similar to border-line representatives of other genera, for example, *Fusarium* and *Cylindrocladium*. Under the circumstances it becomes difficult for investigators who are not thoroughly familiar with these groups to distinguish the representatives of one genus from those of another.

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A STUDY OF VARIOUS FRACTIONS OF *BRUCELLA ABORTUS*

I. PREPARATION, TOXICITY AND BIOCHEMICAL NATURE OF ALCOHOLIC PRECIPITATE[1]

By Ronald Gwatkin[2]

Abstract

Alcoholic precipitates were prepared from shaken and filtered suspensions of *Brucella abortus*. These precipitates were highly toxic for guinea pigs by intraperitoneal injection. The toxic and antigenic fraction was water soluble. Filtration did not modify the toxic effect. Dialysis removed some of the lethal substance in one trial but did not affect the suspension in a subsequent experiment. Varying volumes of alcohol did not affect the toxic and antigenic qualities of the precipitate. Preparation of these precipitates on several occasions gave rise to symptoms simulating undulant fever in a hypersensitive human subject. Preliminary biochemical examination suggested that the precipitate consisted almost entirely of carbohydrates.

Introduction

Rake (4, 5, 6, 7) has reported work on fractions of the meningococcus. One paper (6) deals with the precipitation by alcohol of a solution of ground meningococcus. This solution was filtered. One portion was precipitated with eight volumes of 95% alcohol dissolved in distilled water and reprecipitated until free from non-carbohydrate substance. This was not type nor species specific. Another portion, precipitated with 1½–2 volumes of alcohol and reprecipitated until free from non-carbohydrate substance was classified as the soluble or S-specific substance.

Zozaya (8) in 1931 reported a serological study of the polysaccharides of meningococcus, *B. anthracis*, *B. proteus*, *B. subtilis* and *B. mesentericus*. He found that the meningococcus polysaccharide reacted with a broad precipitable carbohydrate antibody in common with those of the other organisms. Agglutinins had no relation to the carbohydrate precipitable substance, specific or non-specific.

Przesmycki (3) in 1924 published a paper on specific "Residue Antigens" of different types of meningococci. These were prepared as follows: Blake bottles were washed off with 25 cc. of salt solution each. The suspension was shaken five to six hours. Gross particles were removed by centrifugation until an almost clear, opalescent, yellowish fluid was obtained. This was precipitated by the addition of 10% acetic acid drop by drop. Usually but little precipitate was obtained. This was removed by centrifugation. The supernatant fluid was boiled two to three minutes and the precipitate again removed. The reaction was adjusted to pH 7.0 with 10% sodium hydroxide, and to the fluid was added five volumes of absolute alcohol. It was left at room temperature 12 to 16 hr. The precipitate was collected by centrifugation, washed with alcohol and ether and then dried. A very small amount of a grayish-white precipitate was obtained. A suspension of 1 : 100 was made in distilled water by the addition of acetic acid, which was afterwards neutral-

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ized. Serums prepared by injections of meningococci showed that the residue antigens gave specific reactions with homologous serums. Heterologous serums gave little or no reaction.

Huston, Huddleson and Hershey (2) in 1934 published a study of the chemical separation of some cellular constituents of the *Brucella* group of micro-organisms.

The *Brucella* were found to be characterized as a group by the absence of free simple sugars, by the occurrence of non-precipitating polysaccharides only, by a large proportion of water-extractable proteins, and by cell lipides analogous to the conventional types found in higher organisms.

The species of the genus *Brucella* could be differentiated one from another by the relative proportions, rather than kind, of two biologically inactive polysaccharide, and two lipide, constituents. *Br. melitensis* was distinguished further from the other two species by the occurrence of a non-protein, non-polysaccharide, precipitating antigen of a type hitherto undescribed.

Preparation of Alcoholic Precipitate of *Brucella abortus*

Przesmycki's method was closely followed at first and a precipitate was produced from *Br. abortus* with which experimental work was carried on. It was found that certain steps could be omitted or combined without making any difference in the final product. The method finally adopted was as follows: *Br. abortus* was grown for three to four days on liver agar, pH 6.6, in flat bottles. It was washed off with 25 cc. of sterile water to each bottle. The bottles had a surface area of about 25 sq. in. The suspension was then shaken for 24 hr. with glass beads in a revolving shaker. It was then heated in flowing steam for five minutes after the temperature of the fluid reached its maximum. As ordinary methods of centrifugation are unsatisfactory with *Brucella*, the suspension was passed through a Sharples supercentrifuge running at 40,000–50,000 r.p.m. This usually gave a clear fluid or one with only slight cloudiness. This was then filtered through a fine Mandler candle and a crystal-clear product was obtained. Five volumes of 95% ethyl alcohol was added to this filtrate, which was then allowed to stand at room temperature until the next day. A fluffy precipitate settled out overnight. This was collected by centrifugation, washed in a mixture of equal parts of alcohol and ether, again collected by centrifugation, and visible fluid evaporated off in the incubator. This usually took two to three hours, after which the drying was completed *in vacuo* over phosphorus pentoxide. The resultant material was ground in an agate mortar and stored in rubber-stoppered vials. For use in the following experiments the powder was suspended in distilled water by grinding. It did not all go into solution, but as a filtrate was as active as the unfiltered suspension, as will be shown later, it was evident that the active portion was readily soluble. There were always particles in an unfiltered suspension, no matter how finely it had been ground. Acetic acid and sodium bicarbonate were both tried in attempts to dissolve these particles but some always remained. Twenty-five flasks of liver agar (625 sq. in. of surface) yielded about one gram of alcoholic precipitate.

Intraperitoneal Injection of Alcoholic Precipitate in Guinea Pigs

In order to test the toxicity of the various lots of alcoholic precipitate, guinea pigs were given varying doses by intraperitoneal injection. Temperatures were taken and the general condition of the animals observed. As will be seen from the following data some guinea pigs died and others recovered. In the immunity experiments, where several injections were given, there was usually a loss of weight and condition, even though the dosage was small, and some died after a number of sub-lethal injections. At first the required dose was injected in 5 or even 10 cc. of fluid, but latterly in 2.0 cc. It was not filtered before injection, except where this procedure is specified, and, as will be seen, filtration did not affect the final suspension. While the active part was undoubtedly in solution there was a certain amount of undissolved material in fine particles. The precipitate seemed to be more readily suspended in water than in salt solution.

Experiment 1. Intraperitoneal injection of 0.005 gm. of Lot 1. A 350 gm. guinea pig was given an intraperitoneal injection of 0.005 gm. of Lot 1 in 1.0 cc. water. Pre-injection temperature was 104.0° F. One hour later the temperature was 102.4, two hours after injection it was the same, and the following morning it was 103.0. Not much disturbance was shown from this small dose, but an injection of 0.02 gm. proved fatal.

Experiment 2. Intraperitoneal injection of 0.01 gm. of Lot 2. A 400 gm. guinea pig was injected by the intraperitoneal route with 0.01 gm. of Lot 2 in 10 cc. saline. The initial temperature was 104.0° F. Seven hours later the temperature was 98.4 and the guinea pig was greatly distressed. Heart action was slowed and thudding. Breathing quickened. The following morning this animal appeared normal and the temperature was 103.0.

The same precipitate was suspended in saline. Two lots of 0.04 gm. in 10 cc. were left in an ice chest overnight. The following day they were diluted to 20 cc. with saline. One lot was then filtered several times through a micro-filter packed with asbestos. This did not clear the solution which was then passed through a Mandler candle, and a crystal-clear solution the color of pale broth was obtained. Ten cc. of this filtrate and 10.0 cc. of the unfiltered suspension (each containing 0.02 gm.) were injected into the abdominal cavity of guinea pigs. Both animals weighed about 350 gm. Table I shows the temperature of these animals. The first temperature was taken at time of injection.

TABLE I

Temperatures of guinea pigs injected with Lot 2, filtered and unfiltered

—	2:00 p.m.	3:00 p.m.	9:15 p.m.	10:00 a.m.
Filtered	101.0	103.0	100.0	103.8
Unfiltered	102.0	104.6	99.6	103.0

It will be seen that there was no practical difference between the filtered and unfiltered product. General symptoms were the same in both animals; tumultuous, thumping heart action and accelerated breathing. Both recovered.

Experiment 3. Intraperitoneal injection of filtered and unfiltered Lot 4 alcoholic precipitate. Lot 3 of the alcoholic precipitate was lost through breaking of the desiccator while under vacuum. Lot 4 was injected into two guinea pigs, one portion filtered as in the previous experiment and the other portion unfiltered. The dose was 0.02 gm. in 10 cc. of water in each case. Table II shows the temperature changes in these animals.

TABLE II

TEMPERATURE CHANGES PRODUCED BY LOT 4 FILTERED AND UNFILTERED

	Mar. 23			Mar. 24	Mar. 25
	11:30 a.m.	2:00 p.m.	5:30 p.m.	9:00 a.m.	9:00 a.m.
Filtered	102.2	102.0	96.0	96.6	102.6
Unfiltered	102.6	101.4	97.4	101.4	102.0

Both animals showed the same symptoms as in the previous experiment but had recovered the second day after the injection. Again there was no significant difference between the filtered and the unfiltered suspension.

Experiment 4. Effect of dialysis on alcoholic precipitate. One tenth of a gram of Lots 4 and 5 was suspended in 12.5 cc. saline. Six cc. of each suspension was placed in a collodion sac in running tap water. The balance of each sample was held in a test tube in the water so as to be kept under the same conditions. The following day each portion was brought to 10.0 cc. containing 0.05 gm. and four guinea pigs received intraperitoneal injections of 7.0 cc. each (0.035 gm. alcoholic precipitate). Table III gives the temperature reactions of these animals.

TABLE III

TEMPERATURE CHANGES PRODUCED BY DIALYZED AND UNTREATED SUSPENSIONS OF LOTS 4 AND 5

	April 12				April 13				April 14	
	11:00 a.m.	1:00 p.m.	4:00 p.m.	5:30 p.m.	9:00 a.m.	12:00 m.	3:15 p.m.	5:00 p.m.	9:00 a.m.	12:00 m.
LOT 4										
Untreated	102.0	98.0	94.0	94.0	94.0	94.0	dead			
Dialyzed	101.8	99.4	96.0	96.0	102.2	103.0	103.0	103.0	101.4	101.6
LOT 5										
Untreated	102.0	98.0	94.0	94.0	94.0	94.0	94.0	94.0	dead	
Dialyzed	101.6	99.0	97.4	97.0	97.6	98.4	101.4	102.8	102.6	102.6

In this experiment, while all the animals were ill, the two that received the untreated suspension died and the two treated with dialyzed material recovered. The dialyzed suspensions had increased in volume and both sacs were shown to be readily porous for sodium chloride when tested afterwards. The color was not removed from the dialyzed fluids. Dialysis did remove some of the temperature-reducing and lethal qualities of these precipitates. This is clearly shown in Fig. 1.

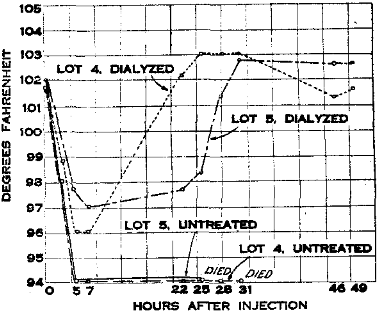

FIG. 1. *Intraperitoneal injections of dialyzed and untreated precipitate. Temperatures of guinea pigs.*

Experiment 5. Intraperitoneal injection of 0.04 and 0.01 gm. of Lot 6 alcoholic precipitate. Two 350 gm. guinea pigs were given intraperitoneal injections of 0.04 and 0.01 gm. of Lot 6 alcoholic precipitate in 5 cc. water. Table IV gives the temperatures of these animals.

TABLE IV

TEMPERATURE OF GUINEA PIGS INJECTED WITH LOT 6 ALCOHOLIC PRECIPITATE

	10:00 a.m.*	11:15 a.m.	1:30 p.m.	3:00 p.m.	4:30 p.m.	9:00 a.m.	12:00 m.	3:30 p.m.	5:00 p.m.	9:00 a.m.
0.04 gm.	103.0	99.4	94.8	94.8	94.0	dead				
0.01 gm.	102.4	102.2	95.0	96.1	97.0	96.2	94.0	94.0	94.0	dead

*Pre-injection temperatures.

The usual symptoms were manifested by these animals. That receiving the larger dose died during the night while the one receiving the smaller dose lived for another day.

Experiment 6. Intraperitoneal injection of Lot 7 alcoholic precipitate. Two guinea pigs weighing about 360 gm. received the same injection as in the previous experiment, namely, 0.04 and 0.01 gm. of Lot 7 alcoholic precipitate in 2 cc. salt solution. Temperatures are shown in Table V.

TABLE V

TEMPERATURES OF GUINEA PIGS INJECTED WITH LOT 7 ALCOHOLIC PRECIPITATE

—	May 18						May 19		May 20
	10:00 a.m.	11:00 a.m.	12:00 m.	2:30 p.m.	3:45 p.m.	4:45 p.m.	9:30 a.m.	11:30 a.m.	10:00 a.m.
0.04 gm.	103.8	102.0	100.4	100.4	100.4	100.0	dead		
0.01 gm.	103.0	103.0	104.8	100.4	98.0	96.6	95.8	94.0	dead

The larger dose did not produce the usual rapid temperature drop but this animal died a day sooner than that receiving the smaller dose.

Experiment 7. Dialysis of Lot 7 alcoholic precipitate. Two lots of 0.05 gm. in 10 cc. water were prepared. One portion was dialyzed in running tap water for 48 hr. The other was immersed in a test tube in the same water. Two guinea pigs were given intraperitoneal injections of 9.0 cc. containing 0.03 gm. They were injected at noon, both temperatures dropped equally rapidly and both animals were dead the following morning. The dialyzing sac was not prepared according to any standard but it was shown to be readily permeable to sodium chloride.

Experiment 8. Intraperitoneal injections of alcoholic precipitate prepared with varying volumes of alcohol. Lot 8 was divided into three portions prior to the precipitating stage and to these were added 2.5, 5 and 10 volumes of 95% ethyl alcohol. The relative weights obtained from 340 cc. of filtrate by each method were: From 2.5 volumes, 0.360 gm.; from five volumes, 0.425 gm.; and from 10 volumes, 0.540 gm. Three guinea pigs were injected with 0.62 gm. of each precipitate in 2 cc. water at 2:00 p.m. All showed the same rapid drop in temperature before night, and all were dead the following morning. Variation from 2.5 to 10 volumes of alcohol made no apparent difference in the temperature-reducing and lethal qualities of the end product. Increase in alcohol did increase the yield of precipitate.

Experiment 9. Intraperitoneal injection of 0.02 gm. of Lot 9 alcoholic precipitate. Two guinea pigs were injected with this lot of alcoholic precipitate. Temperatures are shown in Table VI.

TABLE VI

TEMPERATURES OF GUINEA PIGS INOCULATED WITH LOT 9

—	9:15 *a.m.*	11:15 *a.m.*	1:45 *p.m.*	3:45 *p.m.*	5:00 *p.m.*	12:00 *m.*	9:00 *a.m.*	11:45 *a.m.*	2:00 *p.m.*
1	102.8	100.0	95.0	94.0	dead				
2	103.8	98.2	96.0	97.4	96.0	98.0	97.8	99.2	101.0

One guinea pig died the day of injection. The other was very ill but eventually recovered.

Symptoms and post-mortem findings in guinea pigs. Intraperitoneal injections of very small doses of alcoholic precipitate produced no other effects than a slight elevation of temperature. Larger doses caused nervous symptoms followed by a rapid fall in temperature, loss of power in the limbs, distention of the abdomen and death in from six hours to several days. In those animals that died quickly the only change was a severe inflammation of the peritoneum. In those that lived for a day or more a fibrinous exudate was observed on the liver and a quantity of pus was present. In most cases this was sterile, but from some, colon bacilli, staphylococci and other bacteria were isolated.

Effect of alcoholic precipitate on hypersensitive human subject. It is interesting to note that preparation of this material gave rise to symptoms simulating undulant fever in a hypersensitive person. The subject had a history of what was probably a mild attack of undulant fever some years earlier and had given a positive agglutination and complement fixation reaction in 1 : 100 for the last four years. Tests before this time were not made. The blood showed marked phagocytic activity by Huddleson's method (1). Exposure appeared to desensitize for several weeks. Symptoms were, chill, slight increase of temperature (2°), intense lassitude, double vision in some attacks, pain and stiffness in the small of the back and sometimes in the knee joints, intense sweating during the night, quickened breathing and a thudding heart action. The day after exposure the symptoms abated, leaving a feeling of marked lassitude. These symptoms developed on three occasions, following the grinding or evaporation of products of *Br. abortus* in quantity. The subject is not affected by the ordinary laboratory handling of the organism.

Biochemical Nature of Alcoholic Precipitate

A suspension of 0.02 gm. of Lot 2 in 20 cc. salt solution (1 : 100) was allowed to stand in the refrigerator overnight. It was filtered through a fine Mandler candle. Dr. C. S. Hanes of the Foundation examined this solution and reported as follows:—

Hopkins Cole Test—Faint positive. *Millon's Test*—Negative. *Biuret*—Faint violet.

Dr. Hanes tried to precipitate the remaining fluid with 60% saturated ammonium sulphate solution. It became slightly cloudy but did not advance any further and no precipitate was formed.

A suspension of 0.2 gm. in 50 cc. saline was prepared and filtered through a Mandler candle. Five-cc. lots of the filtrate were treated by Dr. Hanes with ammonium sulphate, trichloracetic acid, trypsin, heat and hydrochloric acid, and heat and sodium hydroxide. The ammonium sulphate produced a precipitate. Trichloracetic acid produced a faint opalescence with 5% and 12% after four hours. Hydrochloric acid and heat did not cause precipitation (5 cc. sol. + 2 cc. *N* hydrochloric acid). Sodium hydroxide and heat (5 cc. sol. + 2 cc. *N* sodium hydroxide) caused a heavy flocculent precipitate. This disappeared on neutralization with hydrochloric acid. Injection experiments, skin tests and complement fixation tests were carried out. The suspension, unfortunately, appeared to have been too weak for its purpose and rather indefinite results were obtained.

Lot 12 precipitate was divided and 0.45 gm. was suspended in 200 cc. distilled water. After about an hour, with intermittent shaking, it was filtered through paper and a fine Mandler candle. Five volumes of 95% alcohol was added to the filtrate, which was allowed to stand overnight. The fluid became cloudy but no precipitate was formed. *N* sodium hydroxide was added and a precipitate began to form in a few minutes. This was allowed to settle out, collected by centrifugation and dried, first in the incu-

bator and then over phosphorus pentoxide *in vacuo*. The supernatant fluid was filtered through a fine candle to remove any particles of precipitate. The first precipitate, the second precipitate, and the residue of the supernatant fluid were turned over to Dr. A. D. Barbour for examination. Guinea pigs were injected with 0.01 gm. of each product in 2.0 cc. water. The original precipitate caused a fall in temperature in the injected guinea pig from 103.0 to 99.8° F. The reprecipitated material, contrary to expectations, was not toxic. There was but slight change in temperature, less than a degree, during the period of observation. The residue of the supernatant fluid caused a slight rise of temperature. This material of course contained the added sodium hydroxide, and other soluble material, so that the actual amount of active material would be less than 0.01 gm. Dr. Barbour's report on the three samples is given in Table VII.

TABLE VII

Test	Given by	1	2	3
		Original precipitate	Repre-cipitated	Residue
Biuret	All proteins	?	—	?
Xanthoproteic	Thyosine Phenylalamine Tryptophane	?	—	+ (weak)
Millon	Tyrosine	—	—	—
Ferrocyanide	All proteins	?	—	?
Molisch	All carbohydrates	++++	++++	++++

Dr. Barbour states: "I would conclude from this that your material consists almost entirely of carbohydrates, with possibly a trace of protein in samples 1 and 3."

A mixture of several lots of alcoholic precipitate was next prepared and 0.45 gm. was ground up in water and the volume brought to 200 cc. This was allowed to stand overnight, filtered, and 10 cc. *N* sodium hydroxide and 1000 cc. alcohol were added. The precipitate that formed was collected and dried, as was also the insoluble material on the filter paper. Guinea pigs were given intraperitoneal injections of these substances and the original precipitate as follows:—

No. 1. 0.015 gm. of insoluble material.

No. 2. 0.02 gm. of reprecipitated material.

No. 3. 0.02 gm. of original precipitate.

The temperature reactions of these animals are shown in Table VIII.

TABLE VIII

TEMPERATURES OF GUINEA PIGS INJECTED WITH PRECIPITATES AND INSOLUBLE MATERIAL

	10:00 a.m.	12:00 m.	2:00 p.m.	4:00 p.m.	5:00 p.m.	9:00 a.m.	12:00 m.	2:00 p.m.	4:00 p.m.	9:00 a.m.	12:00 m.
1	101.6	94.8	98.6	93.0	93.0	93.0	94.0	94.0	94.0	98.4	100.6
2	102.0	93.0	93.0	93.0	93.0	dead					
3	101.8	98.4	100.4	95.0	94.4	96.6	98.6	100.4	100.4	101.4	102.0

The temperatures of all three guinea pigs dropped and all were ill, but those receiving the insoluble material and the original precipitate recovered, while the animal that was injected with the reprecipitated material died.

Summary

An alcoholic precipitate was prepared from a filtrate of *Brucella abortus* suspension. Intraperitoneal injection of this material in guinea pigs produced peritonitis and death where the dosage was sufficiently large.

Seventeen guinea pigs received intraperitoneal injections of untreated alcoholic precipitate ranging from 0.005 to 0.04 gm. Twelve died and five lived. The animal receiving the smallest dose did not show a drop below the normal range of temperature. The others all showed a drop in temperature. Three animals received 0.01 gm. Two of these died. Eight were given 0.02 gm. and in this group five died. The five guinea pigs that received 0.03 to 0.04 gm. all died.

Dialysis reduced the temperature-lowering and lethal fraction in the first experiment with injections of 0.035 gm. but in the second experiment, although the material was dialyzed longer, both animals died from an injection of 0.03 gm. The dialyzing sacs were not standardized in either case.

Filtration of the suspension of alcoholic precipitate through a Mandler candle before injection did not modify the effect on injected guinea pigs, indicating that the active part of the material readily went into solution in water.

The preparation of the precipitate gave rise to symptoms simulating undulant fever in a hypersensitive human subject. The attacks appeared to desensitize the subject for several weeks.

Preliminary biochemical examination suggested that the precipitate consisted almost entirely of carbohydrates. The original precipitate and the residue of the supernatant fluid showed a trace of protein that was absent in reprecipitated material.

Acknowledgments

The writer is indebted to T. Lloyd Jones, B.V.Sc., for his assistance, to Dr. A.D. Barbour, and Dr. C. S. Hanes, for making the biochemical examinations, and to Dr. H. B. Speakman and Dr. S. Hadwen for their advice in connection with this work, which was made possible by a grant from the Department of Agriculture of Ontario.

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A STUDY OF VARIOUS FRACTIONS OF *BRUCELLA ABORTUS*

II. COMPLEMENT FIXATION AND INTRADERMAL TESTS WITH ALCOHOLIC PRECIPITATE[1]

By Ronald Gwatkin[2]

Abstract

Alcoholic precipitates of *Brucella abortus* showed high antigenic qualities in the complement fixation test. Dialysis and filtration did not lower this activity. The precipitates gave rise to reactions in infected guinea pigs, in dilutions which produced no change in normal animals. Filtration did not modify the results. In two out of three samples dialysis did not lower the activity of the suspensions as skin test antigens. The addition of formol did not modify the skin reactions.

Introduction

In a previous paper (1) the writer described the preparation, biochemical nature and toxicity of an alcoholic precipitate prepared from a filtered suspension of *Br. abortus*. This article deals with the precipitate as an antigen in the complement fixation test, and also as an agent for the intradermal test in normal and infected guinea pigs.

Complement Fixation Tests with Alcoholic Precipitate

Complement fixation tests were carried out with the various lots of alcoholic precipitates, untreated, dialyzed and filtered. Details of these are given in the following experiments. In general the suspensions made fairly good antigens, and could be used sufficiently dilute to avoid anticomplementary reactions. At first various strengths were used but latterly 0.01 gm. was suspended in 2 cc. saline (1:200), and from this, dilutions of 1:25, 1:100 and 1:500 were made, which gave final dilutions of 1:5000, 1:20,000 and 1:100,000 of the precipitate. In order to use small quantities of precipitate, 0.01 gm. was suspended in 2 cc. saline. To this was added 48 cc. saline making the 1:25 dilution. Part of this was filtered and then diluted to make the 1:100 and 1:500 dilutions of the suspension. The unfiltered suspension was diluted at the same time. One cc. of each dilution was used in the test, which was the one regularly used in this laboratory for routine diagnosis and experimental work. All alcoholic precipitate antigens were heated just before use for 30 min. at 56° C.

Experiment 1. Complement fixation test on Lot 1. A suspension of 0.1 in 5 cc. was prepared. This was diluted 1:25 and 1:1000 (1:1250 and 1:50,000 of the precipitate). Results are shown in Table I.

[1] *Manuscript received September 27, 1934.*
Contribution from Department of Pathology and Bacteriology, Ontario Research Foundation, Toronto, Canada.
[2] *Research Fellow.*

TABLE I

COMPLEMENT FIXATION TEST OF LOT 1 ALCOHOLIC PRECIPITATE

	1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500	1 : 5000
Alcoholic precipitate 1 : 25	4	4	4	4	4	0	0	0
1 : 1000	0	0	0	0	0	0	0	0
Regular antigen 1 : 25	4	4	4	4	4	4	3	0

In the tables showing results of complement fixation tests: 4 = complete fixation, 3 = 75% fixation, 2 = 50% fixation, 1 = 25% fixation, 0 = no fixation.

The spread between the two dilutions was too great and a 1:50,000 dilution of the precipitate did not produce fixation with the serum employed, which had a titre of 1:1000 by the regular test.

Experiment 2. Complement fixation test on Lots 1 and 2, filtered and unfiltered. A suspension of 0.01 gm. in 2 cc. (1:200) was diluted to 1:25 and 1:100 (dilutions of 1:5000 and 1:20,000 of the precipitate). One portion was filtered through a Mandler candle and the other was left unfiltered. The results of this test are shown in Table II.

TABLE II

COMPLEMENT FIXATION TEST ON LOTS 1 AND 2, FILTERED AND UNFILTERED

	1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
Al. ppt. 1 1 : 25	4	4	4	4	0	0	0
1 : 100	4	4	4	4	4	0	0
Al. ppt. 1 (filtered)							
1 : 25	4	4	4	4	0	0	0
1 : 100	4	4	4	4	4	0	0
Al. ppt. 2 1 : 25	4	4	4	4	4	0	0
1 : 100	4	4	4	4	4	4	0
Al. ppt. 2 (filtered)							
1 : 25	4	4	4	4	4	0	0
1 : 100	4	4	4	4	4	4	0
Regular antigen 1 : 15	4	4	4	4	4	4	0

It will be seen from Table II that the higher dilution of each precipitate gave one tube higher fixation than the lower dilution. Presumably this is due to some inhibitory substance that disappears in the higher dilutions.

Experiment 3. Complement fixation test on Lot 4, filtered and unfiltered. A suspension of 0.01 gm. in 2 cc. was made and the same dilutions prepared as in the previous experiment. The results are shown in Table III.

TABLE III

COMPLEMENT FIXATION TEST ON FILTERED AND UNFILTERED SAMPLES OF LOT 4

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
Filtered	1 : 25	Anticomplementary						
	1 : 100	4	4	4	4	2	0	0
Unfiltered	1 : 25	Anticomplementary						
	1 : 100	4	4	4	2	0	0	0
Regular antigen	1 : 15	4	4	4	4	4	4	0

It will be seen in Table III that, although the difference was slight, the filtered suspension showed more fixation than the unfiltered. Neither gave as high a reaction in comparison with the regular bacterial antigen as did Lot 2 in Table II. This precipitate was anticomplementary in 1:25 dilution of the suspension.

Experiment 4. Complement fixation test on Lots 4 and 5, dialyzed and untreated. One-tenth gm. of each precipitate was suspended in 12.0 cc. saline. Six cc. of each was placed in a collodion sac and dialyzed overnight in running water. The remainder was immersed in a test tube in the water to have the holding conditions the same. Both lots were brought next day to 10 cc., each containing 0.05 gm. Dilutions of 1:25, 1:100 and 1:500 were made from each suspension, representing dilutions of 1:5000, 1:20,000 and 1:100,000 of the precipitate. The results are given in Table IV.

TABLE IV

COMPLEMENT FIXATION TEST ON DIALYZED AND UNTREATED SAMPLES OF LOTS 4 AND 5

	1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
No. 4 Untreated							
1 : 25	Anticomplementary						
1 : 100	4	4	4	1	1	0	0
1 : 500	0	0	0	0	0	0	0
No. 4 Dialyzed							
1 : 25	Anticomplementary						
1 : 100	4	4	4	1	0	0	0
1 : 500	0	0	0	0	0	0	0
No. 5 Untreated							
1 : 25	4	4	4	4	4	0	0
1 : 100	4	4	4	4	4	1	0
1 : 500	3	3	4	4	4	4	0
No. 5 Dialyzed							
1 : 25	4	4	4	4	4	1	0
1 : 100	4	4	4	4	4	2	0
1 : 500	2	3	4	4	4	4	0
Regular antigen 1 : 15	4	4	4	4	4	4	0

Lot 4 was again anticomplementary in the lowest dilution. The highest dilution gave the best fixation with both lots. No. 5 was equal to the bacterial antigen. The dialyzed product was the same as the untreated one, any differences being too slight to be considered. Reference might be made here to another experiment (1), in which these same dialyzed and untreated precipitate suspensions were injected into guinea pigs by the intra-abdominal route. Both dialyzed-suspension animals lived while the untreated-suspension pair died. It will be noticed that Lot 5 shows a prezone phenomenon in the first two tubes.

Experiment 5. Complement fixation test on Lot 6. A suspension of 0.01 in 2 cc. was diluted 1:25, 1:100 and 1:500 to make precipitate dilutions of 1:5000, 1:20,000 and 1:100,000. Results are shown in Table V.

TABLE V

COMPLEMENT FIXATION TEST ON LOT 6

	1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
1 : 25	4	4	4	0	0	0	0
1 : 100	4	4	4	3	0	0	0
1 : 500	4	4	4	4	0	0	0
Regular antigen 1 : 15	4	4	4	4	4	4	0

As with Lots 4 and 5, the best fixation was shown in the highest dilution. This lot did not equal the bacterial antigen with the serum employed.

Experiment 6. Complement fixation test with dialyzed and untreated samples of Lot 7. A suspension of 0.01 gm. in 2.0 cc. was diluted 1:25, 1:100, and 1:500. The results of this test are given in Table VI.

TABLE VI

COMPLEMENT FIXATION TEST ON LOT 7

	1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
1 : 25	4	4	2	2	0	0	0
1 : 100	4	4	4	4	0	0	0
1 : 500	4	4	4	4	4	0	0
Regular antigen 1 : 15	4	4	4	4	4	4	0

The highest dilution again gave the best fixation but did not equal the bacterial suspension antigen.

Two lots of 0.05 gm. of this precipitate were suspended in 10.0 cc. water. One lot was dialyzed and the other left in a test tube immersed in the water. The fluid had increased in the sac to 15.0 cc. at 42 hr., so the suspension in the tube was also brought to this quantity, making both dilutions 1:300. Further dilutions were made from this of 1:15, 1:60 and 1:300 (final dilutions of the precipitate of 1:4500, 1:18,000 and 1:90,000). Results of this test are shown in Table VII.

The lowest dilution (1:4,500) was anticomplementary. A dilution of 1:5000 of this precipitate a week previously had not been anticomplement-

TABLE VII

COMPLEMENT FIXATION TEST ON DIALYZED AND UNTREATED SAMPLES OF LOT 7

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000
Dialyzed	1 : 15	Anticomplementary					
	1 : 60	4	4	4	0	0	0
	1 : 300	4	4	4	4	0	0
Untreated	1 : 15	Anticomplementary					
	1 : 60	4	4	4	0	0	0
	1 : 300	4	4	4	3	0	0
Regular antigen	1 : 15	4	4	4	4	4	0

ary. It is possible that this tendency might have developed while the material was in suspension for two days. The previous lot was suspended just before it was required. Both had been heated just before use for 30 min. at 56° C. There was no real difference between the dialyzed and untreated material, and what slight difference there was favored the dialyzed material.

Experiment 8. Complement fixation test on Lots 8, 9, 10 and 11. Lot 8 was divided into three portions and precipitated with 2.5, 5 and 10 volumes of alcohol. Lot 9 was made in the ordinary way with five volumes of alcohol as were also Lots 10 and 11. Suspensions of 0.01 gm. of precipitate were treated in the ordinary way and results are shown in Table VIII.

TABLE VIII

COMPLEMENT FIXATION TEST ON LOTS 8, 9, 10 AND 11

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500	1 : 5000
Lot 8 (2.5 vols.)	1 : 25	Anticomplementary							
	1 : 100	4	4	4	3	0	0	0	0
	1 : 500	4	4	4	4	0	0	0	0
Lot 8 (5 vols.)	1 : 25	Anticomplementary							
	1 : 100	4	4	4	0	0	0	0	0
	1 : 500	4	4	4	4	0	0	0	0
Lot 8 (10 vols.)	1 : 25	Anticomplementary							
	1 : 100	4	4	4	0	0	0	0	0
	1 : 500	4	4	4	4	0	0	0	0
Lot 9	1 : 25	4	4	4	3	0	0	0	0
	1 : 100	4	4	4	4	0	0	0	0
	1 : 500	4	4	4	4	4	1	0	0
Regular antigen	1 : 15	4	4	4	4	4	4	0	0
Lot 10	1 : 25	4	4	4	4	4	0	0	0
	1 : 100	4	4	4	4	4	0	0	0
	1 : 500	4	4	4	4	4	4	0	0
Lot 11	1 : 25	4	4	4	4	2	0	0	0
	1 : 100	4	4	4	4	4	2	0	0
	1 : 500	4	4	4	4	4	3	0	0
Regular antigen	1 : 15	4	4	4	4	4	4	4	0

There was very little difference between the precipitates produced by 2.5, 5 and 10 volumes of alcohol. The highest dilution in each case gave identical results and the lowest was anticomplementary. In all lots the best fixation was shown by the highest dilution of the precipitate.

Intradermal Tests with Alcoholic Precipitate

Infected and normal guinea pigs and rabbits were tested with suspensions of alcoholic precipitate to determine the skin response in these animals. It is realized that skin tests in small animals may not be applicable to cows, but they were carried out for the purpose of obtaining as much information as possible about the antigenic qualities of these precipitates.

Experiment 9. Intradermal test of infected and normal guinea pigs with Lot 1 alcoholic precipitate. Two infected and two normal guinea pigs were shaved over the abdominal region. Each guinea pig was injected with 0.1 cc. of a suspension of 0.005 gm. in 1.0 cc. saline (1:200). The following day the infected guinea pigs showed a small but distinct inflammatory reaction. The normal animals showed no reaction. The swelling on the positive animals persisted for several days.

Experiment 10. Intradermal test of normal and infected guinea pigs with filtered and unfiltered suspensions of Lot 2 alcoholic precipitate. A suspension of 0.01 gm. in 10.0 cc. saline (1:1000) was prepared and intradermal injections were made in the shaved abdominal region. At 19 hr. there was a slight reaction in the infected animal. The reaction was quite distinct at 24 hr. and persisted for several days. There was no reaction in the normal guinea pig.

A suspension of 0.01 gm. in 2 cc. (1:200) produced a well-marked swelling in another infected guinea pig but also caused a reaction in a normal animal.

A suspension of 0.02 gm. in 10.0 cc. saline (1:500) was prepared. Half of it was filtered through a fine Mandler candle. A normal and an infected guinea pig were injected intradermally in the abdominal region with 0.1 cc. of filtered and unfiltered suspension. Both produced a reaction in the infected animal that was observable at 7 hr. and well marked at 24, 48 and 72 hr. Neither product produced any reaction in the normal animal.

Experiment 11. Intradermal test with Lot 4, filtered and unfiltered. A suspension of 0.02 gm. in 10.0 cc. saline (1:500) was divided, and one portion was filtered through a fine Mandler candle. Each was injected in the shaven skin of the abdomen of a normal and an infected guinea pig. In six hours there was a reaction to both filtered and unfiltered suspensions in the infected guinea pig but not in the normal animal. This condition was the same at 24 and 48 hr.

Experiment 12. Intradermal tests of rabbits with Lots 4 and 5, dialyzed and untreated. One-tenth of a gram of each lot was suspended in 12.0 cc. salt solution. Six cc. of each suspension was placed in a collodion sac in running

tap water. The other 6 cc. was held in a test tube in the same water, in order to have keeping conditions as close as possible. The following day both lots were diluted to 10.0 cc. with saline (1:500). A normal and an infected rabbit were clipped and shaved over the abdominal area. One-tenth cc. of the dialyzed and untreated suspensions was injected into each rabbit. At 24 hr. all the suspensions showed a clear reaction, but untreated Lot 4 was very prominent. Pus appeared in this lesion at 48 hr. while the reaction from the dialyzed No. 4 sample had practically disappeared. At this time both dialyzed and untreated suspensions of Lot 5 showed a distinct reaction, no difference being observed between the two. *E. coli* was recovered from the untreated No. 4 lesion. The marked reaction was obviously due to this cause. Dialysis made no difference to Lot No. 5. The reaction from the dialyzed No. 4 sample had practically disappeared at 48 hr. but this could not be compared to the undialyzed portion on account of infection in that reaction site.

Experiment 13. Intradermal tests of guinea pigs with Lot 7, dialyzed and untreated. A dilution of 1:300 of dialyzed and untreated alcoholic precipitate was injected in an infected and a normal guinea pig. One-tenth cc. of each was injected in the shaven skin of the abdomen. The suspension had been dialyzed in running tap water for 48 hr. At 24 hr. there was some reaction from both suspensions in both the normal and infected guinea pigs. The swelling disappeared in the former but there were small, hard lumps from both dialyzed and untreated material on the infected pig four days after injection. Dialysis did not make any appreciable difference to the reaction of this material.

Experiment 14. Formolized and untreated suspensions of Lot 7. A suspension was made of 0.05 gm. of Lot 7 in 10.0 cc. water (1:500), which was divided into two lots. To one was added 0.25% of formol C.P. Both were held in the refrigerator for two days. One-tenth cc. of each was injected into the shaven skin of a normal and an infected guinea pig. There was no difference between the reaction of the formolized and untreated filtrate in the infected guinea pig and neither produced reactions in the negative animal.

Summary

Alcoholic precipitate of *Br. abortus* showed high antigenic qualities in the complement fixation test. Dilutions of 1:100,000 as a rule gave the best fixation, although there were some exceptions. Some of the precipitates were anticomplementary in a dilution of 1:5000. Dialysis did not lower the antigenic quality of a suspension of precipitate. The collodion sacs were not standardized but were readily permeable to sodium chloride. Filtering through a fine Mandler candle did not lower the antigenic quality of a suspension of precipitate. Precipitation of the filtrate with 2.5, 5 and 10 volumes of alcohol did not modify the precipitate as an antigen in the complement fixation test.

The intradermal injection of alcoholic precipitate gave rise to an inflammatory reaction in infected guinea pigs in dilutions of 1:500 and 1:1000.

These dilutions did not give rise to reactions in normal animals. One of two lots of precipitate diluted 1:200 caused an inflammatory reaction in a normal guinea pig, as did also a 1:300 dilution of another lot.

Filtering through a fine Mandler candle did not affect the activity of a suspension as an antigen in the skin test.

Skin reactions were not influenced by dialysis of two out of three lots of precipitate. The third sample appeared to have been reduced in activity but no comparison could be made with the injection of untreated suspension as infection occurred at this site of injection.

The addition of 0.25% formalin did not influence the activity of a sample of precipitate as an antigen in the skin test.

Acknowledgments

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Reference

1. GWATKIN, RONALD. A study of various fractions of *Brucella abortus*. I. Preparation, toxicity and biochemical nature of alcoholic precipitate. Can. J. Research, 12: 115-124. 1935.

A STUDY OF VARIOUS FRACTIONS OF *BRUCELLA ABORTUS*

III. IMMUNITY EXPERIMENTS WITH ALCOHOLIC PRECIPITATE[1]

By Ronald Gwatkin[2]

Abstract

Brucella antiserum protected guinea pigs against intraperitoneal injections of alcoholic precipitate. After absorption with *Brucella abortus* this serum was not protective. Normal serum also failed to modify the toxicity of the precipitate. Alcoholic precipitate was as efficient as whole culture in removing antibodies *in vitro*, as judged by serological tests. Intraperitoneal injections of precipitate in small animals stimulated agglutinin production and opsonocytophagic activity, but did not protect guinea pigs against infection by eye with *Brucella abortus* or prevent abortions in infected animals. Oral administration of precipitate did not give rise to symptoms in guinea pigs.

Introduction

In previous papers (2, 3) the writer has described the preparation of an alcoholic precipitate from a filtered suspension of *Br. abortus*, the biochemical nature of the product, its use as an antigen in the complement fixation test and in skin tests on normal and infected guinea pigs and rabbits.

This article records the effect of anti-abortus serum in neutralizing the toxicity of the precipitate, oral administration of precipitate, and its use as an antigen to produce active immunity in guinea pigs.

Effect of Anti-abortus Serum on *Br. abortus* Alcoholic Precipitate

An alcoholic precipitate was obtained from a colon bacillus. This was even more toxic for guinea pigs than the *Brucella* precipitate. In view of this it was necessary to test the specificity of the *Brucella* product, which was done by protection tests of guinea pigs with anti-abortus serum from various animals.

Experiment 1. In this experiment an old horse serum was employed. It had been preserved with merthiolate 1 : 5000. It was filtered through a Mandler candle before use. It was found too late that this serum had developed a temperature-reducing quality and it was therefore unsuitable for the purpose, and did not protect the injected guinea pigs.

Experiment 2. Fresh citrated rabbit blood. Citrated blood from rabbits that had received several injections of *Br. abortus* was collected. Guinea pigs were given intraperitoneal injections as follows:—

No. 1. 5 cc. whole, citrated blood.

No. 2. 5 cc. whole, citrated blood + 0.04 gm. Lot 5 precipitate.

No. 3. 5 cc. water + 0.04 gm. Lot 5 precipitate.

[1] *Manuscript received September 27, 1934.*
Contribution from Dept. of Pathology and Bacteriology, Ontario Research Foundation, Toronto, Canada.
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Table I shows the temperatures of these animals. The first, at noon, is the pre-injection temperature.

TABLE I

TEMPERATURES OF GUINEA PIGS IN EXPERIMENT 2

	April 17			April 18			April 19–20		
	12:00 m.	2:30 p.m.	5:15 p.m.	10:00 a.m.	12:30 p.m.	5:00 p.m.	10:00 a.m.	4:45 p.m.	9:00 a.m.
No. 1	102.6	95.0	97.0	102.8	103.0	102.2	102.0	102.0	102.0
No. 2	102.0	94.0	94.0	102.0	103.4	104.4	102.8	102.4	102.2
No. 3	103.0	94.0	96.4	94.0	94.0	94.0	100.0	101.0	102.0

Rabbit blood alone caused some drop in temperature. The temperature of the blood-and-precipitate animal dropped to 94° but rose to normal the next day. Most of the animals that recover from injections of precipitate show an increase above normal temperature following the subnormal effect. The precipitate-alone animal remained low for a day longer than the others but all three recovered.

Experiment 3. Effect of intraperitoneal injections of water, sodium citrate and broth in guinea pigs. It was considered necessary at this time to try the effect of water, sodium citrate solution and broth by intraperitoneal injection. No. 1 was given 5 cc. tap water. No. 2 was given 5 cc. tap water containing 0.1 gm. sodium citrate and No. 3 was injected with 5 cc. beef broth, pH 7.8. The temperatures of these animals are given in Table II.

TABLE II

TEMPERATURES OF GUINEA PIGS INJECTED WITH WATER, SODIUM CITRATE AND BROTH

	10:00 a.m.	12:30 p.m.	3:30 p.m.	5:00 p.m.	10:00 a.m.
No. 1	102.0	102.0	102.2	102.8	102.6
No. 2	101.0	102.0	101.4	101.8	101.6
No. 3	103.0	103.2	103.0	102.4	103.0

These guinea pigs showed no bad effects from the injections and the temperatures remained within normal range.

Experiment 4. Effect of heated rabbit serum on alcoholic precipitate. Serum from the rabbits mentioned in Experiment 2 was heated at 56° C. for 30 min. As in the former experiment, three guinea pigs were injected as follows:—

No. 1, weight 325 gm. 5 cc. heated serum alone.

No. 2, weight 312 gm. 5 cc. heated serum + 0.04 gm. Lot 5.

No. 3, weight 330 gm. 5 cc. water + 0.04 gm. Lot 5.

The temperatures of these animals are given in Table III and also the difference is very clearly shown in Fig. 1.

TABLE III

TEMPERATURES OF GUINEA PIGS INJECTED WITH SERUM AND ALCOHOLIC PRECIPITATE

	1:30 p.m.	4:45 p.m.	9:30 a.m.	12:00 m.	5:00 p.m.	9:30 a.m.
No. 1	102.0	101.6	101.8	102.2	101.6	101.8
No. 2	101.6	97.0	100.6	100.4	103.2	103.0
No. 3	102.0	95.0	94.0	dead		

The temperature of the serum-alone guinea pig did not go out of the normal range. The temperature of the serum-and-precipitate animal dropped to 97° but rapidly returned to normal. The precipitate-alone guinea pig died. Postmortem examination of No. 3 showed peritonitis and fibrinous exudate over liver. Cultures were negative. Results were clear-cut in this experiment because there were no harmful results from the serum, which had been heated to remove its hemolytic activity.

Experiment 5. Hemolytic action of rabbit serum on guinea pig erethrocytes. Serums from the rabbits employed in Experiments 2 and 4 were tested for hemolytic activity on guinea-pig cells. In our earlier work (1, 4) it was found that fresh bovine serum was hemolytic and could not be safely injected into guinea pigs. The same procedure was employed. One-half cc. of each rabbit serum was added to 1.0 cc. of a 1% suspension of washed guinea-pig red cells and placed in a 37° C. water bath. The same quantity of these serums heated for 30 min. at 56° C. was added to 1.0 cc. quantities of cell suspension, and a tube of cell suspension only was included. The serum of the three rabbits which had been used for the protection test produced complete hemolysis of the guinea-pig cells in two minutes. Serum from another rabbit produced slight hemolysis in four minutes, but it was never complete. The bad effects from the use of the fresh, unheated rabbit serum were evidently due to hemolytic activity.

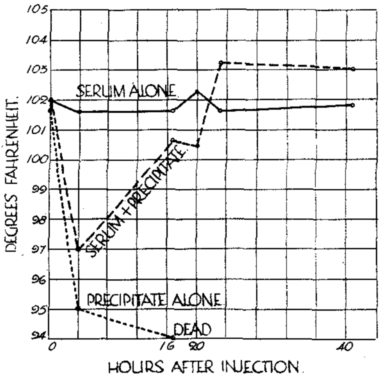

Fig. 1. Effect of heated rabbit serum on alcoholic precipitate. Temperatures of guinea pigs.

Experiment 6. Effect of heated normal and immune bovine serum on alcoholic precipitate. A mixture of Lots 3, 4, and 5, alcoholic precipitate was used.

The following suspensions were made and placed in the refrigerator overnight.

1. 0.04 gm. al. ppt. + 1.0 cc. pos. serum + 4 cc. water.
2. 0.04 gm. al. ppt. + 5.0 cc. pos. serum.
3. 0.04 gm. al. ppt. + 1.0 cc. neg. serum + 4 cc. water.
4. 0.04 gm. al. ppt. + 5.0 cc. neg. serum.
5. 0.04 gm. al. ppt. + 5.0 cc. water.
6. 5.0 cc. positive serum.
7. 5.0 cc. negative serum.

The positive serum was obtained from a cow that had been a confirmed reactor for several years. It had an agglutinin titre of 1 : 1000. The serum was heated for 30 min. at 56° C. before the suspensions were made up. Temperatures are given in Table IV.

TABLE IV

TEMPERATURES OF GUINEA PIGS IN EXPERIMENT 6

—	10:00 a.m.	11:00 a.m.	1:30 p.m.	3:00 p.m.	4:30 p.m.	9:00 a.m.	12:00 m.	3:30 p.m.	5:00 p.m.	9:00 a.m.
1	103.0	99.2	95.8	94.8	94.0	94.0	95.8	98.4	100.2	102.0
2	103.2	100.8	98.2	95.8	95.4	101.6	102.4	103.4	102.4	102.2
3	103.0	100.0	94.0	94.0	95.2	94.0	94.0	94.0	94.0	102.0
4	103.0	98.7	94.4	94.0	94.0	94.0	94.0	94.0	94.0	dead
5	103.0	99.0	98.2	97.1	97.0	94.0	94.0	100.6	101.0	103.2
6	102.5	101.2	101.2	102.8	102.4	102.4	102.4	102.4	102.8	
7	103.8	102.0	100.0	100.0	101.2	102.4	103.4	103.0	103.2	

In this experiment positive serum was effective in preventing the temperature fall to some extent, and in more rapid recovery. Nos. 3 and 5 nearly died, but eventually recovered. The normal serum did not modify the action of the precipitate.

Experiment 7. Effect of absorbed and unabsorbed positive serum on intraperitoneal injections of precipitate. The positive serum was that employed in Experiment 6. The negative serum was obtained by pooling routine samples of blood. Both lots were heated for 30 min. at 56° C. Two cc. of packed *Br. abortus* was added to 10 cc. of positive serum. This and 10 cc. of negative serum were allowed to stand at room temperature for two days. Both serums contained 0.5% phenol as preservative. The absorbed tube was centrifuged and the serum drawn off. One guinea pig was given an intraperitoneal injection of 5.0 cc. absorbed serum and the other 5.0 cc. of untreated serum. Both serums had suspended in them 0.02 gm. of alcoholic precipitate. Fig. 2 shows the temperature reactions following the injections. The difference is very striking and is further confirmation of the specificity fo the alcoholic precipitate.

Agglutination and complement fixation tests were run on the untreated and absorbed serum and also on another tube of the same serum absorbed with alcoholic precipitate. There was not sufficient of this material to absorb a large enough quantity of serum for guinea pig injection so it was tested by the serological tests only. The results are given in Table V.

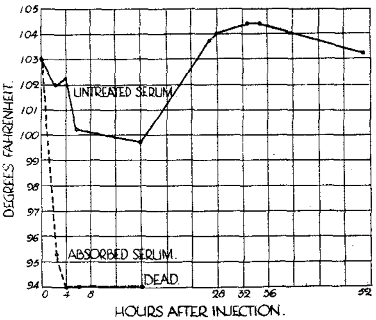

Fig. 2. Effect of absorbed and untreated immune serum on precipitate. Temperatures of guinea pigs.

This experiment showed that alcoholic precipitate was able to absorb an anti-abortus serum equally as well as the whole organism. It further showed that a serum absorbed with the whole organism afforded no protection against the alcoholic precipitate.

TABLE V

AGGLUTINATION AND COMPLEMENT FIXATION TESTS ON ABSORBED SERUMS

			1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
Absorbed with al. ppt.	24 hours	Agglutination	+	—	—	—	—	—	—
		Comp. fixation	4	1	0	0	0	0	0
	48 hours	Agglutination	+	—	—	—	—	—	—
		Comp. fixation	4	0	0	0	0	0	0
Absorbed with *Br. abortus*	24 hours	Agglutination	+	—	—	—	—	—	—
		Comp. fixation	4	4	0	0	0	0	0
	48 hours	Agglutination	+	—	—	—	—	—	—
		Comp. fixation	4	4	0	0	0	0	0
Not absorbed		Agglutination	+	+	+	+	+	+	—
		Comp. fixation	4	4	4	4	4	4	4

+ = Complete agglutination; 4 = Complete fixation; 1 = 25% fixation; 0 = No fixation.

Effect of Alcoholic Precipitate on Agglutinin Production and Phagocytosis, and Result of Oral Administration

Experiment 8. Production of agglutinins by alcoholic precipitate. Four guinea pigs that had survived injections of alcoholic precipitate were tested for presence of agglutinins. Details are given in Table VI.

TABLE VI

AGGLUTININ PRODUCTION OF GUINEA PIGS INJECTED WITH ALCOHOLIC PRECIPITATE

No.	Date of injection	Dose	Date bled	Agglutination		
				1 : 25	1 : 50	1 : 100
1	May 10	0.04 gm.	May 25	—	—	—
2	May 10	0.04 gm.	May 25	—	—	—
3	May 14	0.01 gm.	May 25	—	—	—
4	April 17	0.04 gm.	May 25	+	+	—

Only one of these guinea pigs showed agglutinins at time of bleeding. This was 39 days after it had been injected. The other three were bled 15 and 11 days after injection.

Agglutinin production was clearly shown in the immunity experiments which will be described later. In one case, 12 guinea pigs, after receiving six injections of alcoholic precipitate, had agglutinin titres varying from 1 : 25 to 1 : 250.

Experiment 9. Opsono-cytophagic activity produced in rabbit by injections of alcoholic precipitate. Blood was collected from a normal rabbit, 1 cc. being drawn from the heart and placed in a tube containing 0.05 cc. of 20% sodium citrate. Following the method of Huddleson *et al*, (5) for study of the opsono-cytophagic reaction, 0.1 cc. of citrated blood and 0.1 cc. of a suspension of *Br. abortus* were mixed and incubated for 30 min. at 37° C. Smears were made and stained with Giemsa. There was no phagocytosis. The remaining citrated blood was centrifuged and the agglutination test was set up. There was no agglutination. The rabbit was then given an intraperitoneal injection of 0.04 gm. of alcoholic precipitate in 2 cc. water. At 10:00 a.m., the time of injection, the rabbit's temperature was 102.8° F. At 2:00 p.m. and 4:30 p.m. it was 104.0. The following morning the temperature was 102.6. The rabbit showed no symptoms following this injection.

One week later the rabbit was again bled. Agglutination occurred in a dilution of 1:25. There was marked phagocytosis in all the polymorphonuclear leucocytes examined. At two and three weeks after injection the agglutinin titre was still 1:25 and phagocytic activity was still marked. At one month the agglutination test was negative in 1:25 but phagocytic activity persisted. At five weeks phagocytic activity was again marked, all polymorphonuclear cells being crowded with bacteria. The agglutination test was again negative.

Experiment 10. Effect of alcoholic precipitate by mouth. Two guinea pigs were fasted overnight. In the morning each was given 0.1 gm. of alcoholic precipitate by mouth, mixed in a small quantity of water. The initial temperatures seemed to be lower than normal, which may have been due to fasting. The temperatures of these two guinea pigs are shown in Table VII.

TABLE VII

TEMPERATURES OF GUINEA PIGS RECEIVING ALCOHOLIC PRECIPITATE BY MOUTH

	10:00 a.m.	12:15 p.m.	2:10 p.m.	4:00 p.m.	9:00 a.m.	12:00 m.	2:30 p.m.	4:00 p.m.
No. 1	101.6	102.4	102.6	103.0	102.6	102.0	102.6	103.4
No. 2	101.0	101.6	102.4	102.2	101.2	102.0	101.8	102.6

These guinea pigs showed no bad effects from ingestion of 0.1 gm. alcoholic precipitate.

Immunity Experiments in Guinea Pigs with Alcoholic Precipitate

Experiment 11. Exposure to infection after six injections of alcoholic precipitate. Four guinea pigs were given six intraperitoneal injections of 0.01 gm. of Lot 4 alcoholic precipitate in 2 cc. saline over a period of two weeks. These animals and two normal guinea pigs were exposed to infection by eye with a suspension of *Br. abortus* one week after the last injection. Agglutination tests were made on the blood of these animals at the time when they were exposed, two weeks later, and then at weekly intervals until they were killed nine weeks after commencement of the experiment. There was no significant difference in post-mortem appearance of the organs of the vaccinated animals and controls and *Br. abortus* was recovered from all the spleens. Nos. 2, 3 and 4 had agglutinin titres of 1 : 500, 1 : 50 and 1 : 250, respectively, at the time they were exposed to infection. No. 1 was negative at this time and did not show agglutinins until the controls were positive. There was no evidence of protection in those animals that had been exposed to infection one week after the last of six injections of alcoholic precipitate.

Experiment 12. Exposure to infection by eye at varying intervals after six injections of alcoholic precipitate. Twenty large guinea pigs were selected. They were given an intraperitoneal injection of 0.0025 gm. of Lot 6 alcoholic precipitate in 2.0 cc. water. Temperatures were taken on the first four animals to determine the effect of the injection. There was a drop of only 1 to 1.5° F. The second injection was 0.005 gm. and again there was no disturbance of any account. The third injection was 0.01 gm. The fourth was the same as the third. The fifth was 0.015 gm. No trouble had been experienced up to this time, but following the sixth injection of 0.02 gm. all became very ill and twelve died, leaving only eight for the experiment. Injections had been given at five-day intervals with the exception of one, which was given at four days. There did not seem to be any mounting resistance against the alcoholic precipitate, as the 0.02-gm. injection was not tolerated any better than it would be by animals that had received no previous injections.

One week after the last injection the first three animals, Nos. 2, 3 and 4, were exposed to infection by eye with one drop of *Br. abortus* suspension of

a density equal to 1.0 cc. on the Gates nephelometer. Three normal guinea pigs, Nos. 21, 22 and 23, were similarly exposed at this time. Two weeks after the last injection, the next three survivors, Nos. 6, 9 and 10, were exposed in the same manner with three normal animals, Nos. 24, 25 and 26. Four weeks after the last injection the last two vaccinated animals were also exposed to infection with two normal controls, Nos. 27 and 28. These animals were bled at the time the first group was exposed to infection and then weekly until killed. The first group was killed five weeks, the second four weeks, and the third group 25 days after exposure to infection, as at that time their agglutinin titres clearly indicated that they were infected. The agglutinin titres are given in Table VIII.

TABLE VIII

AGGLUTININ TITRES OF GUINEA PIGS IN VACCINATION EXPERIMENT

No.	May 14	June 14	June 21	June 28	July 5	July 12	July 19	July 27	July 30
2	—	1 : 25	—	1 : 500	1 : 500	1 : 1000	1 : 1000		
3	—	1 : 25	1 : 50	1 : 500	1 : 500	1 : 1000	1 : 1000		
4	—	1 : 200	1 : 100	1 : 100	1 : 500	1 : 1000	1 : 1000		
6	—	—	—	1 : 25	1 : 100	1 : 500	1 : 1000		
9	—	—	—	1 : 25	1 : 250	1 : 1000	1 : 1000		
10	—	1 : 250	1 : 250	1 : 50	1 : 1000	1 : 1000	1 : 1000		
14	—	—	1 : 50	1 : 100	—	1 : 25	died		
18	—	—	1 : 50	1 : 25	—	1 : 25	1 : 100	1 : 1000	1 : 1000
21		—	—	1 : 500	1 : 500	1 : 1000	1 : 1000		
22		—	—	1 : 100	1 : 250	1 : 500	1 : 1000		
23		—	—	1 : 500	1 : 1000	1 : 1000	1 : 1000		
24			—	—	1 : 100	1 : 250	1 : 500		
25			—	—	1 : 100	1 : 100	1 : 250		
26			—	—	1 : 100	1 : 100	1 : 250		
27					—	—	1 : 50	1 : 100	1 : 100
28					—	—	1 : 100	1 : 500	1 : 1000

There was nothing in the agglutination results suggestive of protection. Four guinea pigs had agglutinins in their blood one week after the last injection. These declined and then picked up again following exposure to infection. Table IX gives post-mortem and cultural results on the vaccinated animals and their controls.

No. 14 died during the night of July 16–17 and was badly decomposed. Cultures were overgrown and the presence or absence of *Br. abortus* could not be determined. Death was probably due to the results of the alcoholic precipitate injections. We have had a certain number of guinea pigs that have lived for a considerable length of time after injections which finally proved fatal.

In this experiment, vaccinated animals and controls were alike infected. With the exception of No. 2, however, the spleens of the vaccinated animals were normal in appearance, whereas the control spleens were larger and all showed nodules. This difference was slight but it did suggest that some benefit was derived from the vaccinal injections. The weights of these

TABLE IX

POST-MORTEM AND CULTURAL RESULTS

No.	Post-mortem findings	Wt. of spleen, gm.	Cultures
2	Large spleen	1.3	+
3	Normal spleen	0.8	+
4	Normal spleen	0.75	+
6	Normal spleen	0.8	+
9	Normal spleen	0.85	+
10	Normal spleen	0.7	+
14	Died. Spleen normal. Decomposed		
18	Normal spleen	0.8	+
21	Large nodular spleen	1.6	+
22	Large nodular spleen	1.2	+
23	Large nodular spleen	2.3	+
24	Spleen enlarged. Nodular	1.1	+
25	Spleen not much enlarged but nodular	0.9	+
26	Spleen not much enlarged but nodular	0.85	+
27	Nodular spleen	0.9	+
28	Enlarged, nodular spleen	1.4	+

animals were not of value in forming an opinion, as the injections of precipitate had caused much loss in weight in the group.

Experiment 13. Injection of young sows with alcoholic precipitate prior to breeding. In view of the slight suggestion that some resistance may have developed in the vaccinated animals of Experiment 12, it was decided to vaccinate a group of sows with alcoholic precipitate prior to breeding, infect them at the time the males were added, and note any difference in the production of living young by these animals.

Fifteen young sows were isolated and given six injections of alcoholic precipitate (a mixture of several lots) at five-day intervals. Dosage was kept lower than in previous experiments, but in spite of this, three animals died, leaving 12 for test. Intraperitoneal injections were given in 2 cc. water, the quantity of precipitate received by each pig being approximately as follows:—

First injection	0.0023 gm.	Fourth injection	0.004 gm.
Second injection	0.0026 gm.	Fifth injection	0.003 gm.
Third injection	0.003 gm.	Sixth injection	0.003 gm.

Following the fourth injection all appeared ill and one guinea pig died. Two more died later. It is therefore obvious that maximum doses had been given. It would have been possible to have given larger doses by treating the precipitate with specific serum, but to avoid introducing other factors this was not tried. Injections were commenced June 13 and completed July 9.

On July 12, the 12 surviving vaccinated animals were divided into two lots and two groups of controls were arranged as follows:—

Group 1. Six vaccinated sows and one normal boar.

Group 2. Six normal sows and a boar.

Group 3. Six vaccinated sows and one normal boar.

Group 4. Six normal sows and a boar.

Groups 1 and 2 were exposed to infection by eye on this date with a drop of *Br. abortus* suspension equal in density to 1.0 cc. on the Gates nephelometer. The groups were bled on July 13 and the agglutination test carried out on their serum. The agglutination results are given in Table X.

Group 1 was arranged to show whether vaccinated sows that had been exposed to infection would give birth to living young. Group 2 was a control on this group to show the difference between normal and vaccinated sows that had been exposed to infection. Group 3 was to show whether vaccination with alcoholic precipitate would have any harmful effect on these sows. Group 4 was a control on Group 3, so that comparison might be made between vaccinated and unvaccinated animals which had not been exposed to infection.

TABLE X

AGGLUTINATION TESTS ON GROUPS 1, 2, 3 AND 4

Group	No.	July 13	July 20	July 27	Aug. 2	Aug. 23	Sept. 6	Oct. 29
No. 1 Vaccination and infection	1	1 : 50	1 : 50	1 : 500	1 : 500	1 : 100	1 : 500	1 : 1000
	2	1 : 100	1 : 100	1 : 500	1 : 1000	1 : 1000	1 : 1000	dead
	3	1 : 100	1 : 100	1 : 100	1 : 1000	1 : 1000	1 : 1000	1 : 1000
	4	1 : 100	1 : 100	1 : 500	1 : 1000	1 : 1000	1 : 1000	1 : 1000
	5	1 : 25	1 : 50	1 : 100	1 : 1000	1 : 1000	1 : 1000	1 : 1000
	6	1 : 100	1 : 50	1 : 50	1 : 1000	1 : 1000	1 : 1000	1 : 1000
	*7	—	—	—	—	—	1 : 25	†—
No. 2 Infection only	8	—	—	1 : 50	1 : 100	1 : 1000	1 : 500	1 : 500
	9	—	—	1 : 50	1 : 100	1 : 250	1 : 250	1 : 1000
	10	—	—	1 : 100	1 : 100	1 : 500	1 : 500	1 : 250
	11	—	—	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000
	12	—	—	1 : 100	1 : 100	1 : 500	1 : 250	dead
	13	—	—	1 : 25	1 : 100	1 : 500	1 : 250	1 : 1000
	*14	—	—	—	—	—	—	†—
No. 3 Vaccine only	15	1 : 250	1 : 250	1 : 100	1 : 100	1 : 50	1 : 50	1 : 25
	16	1 : 100	1 : 50	1 : 25	1 : 25	—	—	—
	17	1 : 50	1 : 50	—	—	—	—	—
	18	1 : 50	1 : 25	—	—	—	—	—
	19	1 : 100	1 : 100	—	—	—	—	—
	20	1 : 50	1 : 50	1 : 50	1 : 25	—	—	—
	*21	—	—	—	—	—	—	—
No. 4 Normal	22	—	Not tested					—
	23	—						—
	24	—						—
	25	—						—
	26	—						—
	27	—						—
	*28	—						—

**Boars.* †*New boars.*

The agglutinins shown by Groups 1 and 3 on July 13 were due to the vaccinal injections of alcoholic precipitate. Two weeks later the infected controls had agglutinins in their blood. These antibodies were disappearing in Group 3, vaccine only, and were increasing in Group 1, vaccination and infection. Some reaction persisted in one of the former group until the end of the experiment. From the two previous experiments it was to be expected that the vaccinated animals would become infected.

The two males in Groups 1 and 2 were killed on September 13 and were replaced by new boars. This was done to avoid handicapping these groups through the use of possibly inefficient males. Post-mortem examination, however, did not show any evidence of infection. The serum of No. 7 had an agglutinin titre of 1 : 25 and that of No. 14 was negative.

Groups 1 and 2 were killed on October 29. Each group consisted of five sows and a boar, one sow in each group having died. All the young had been aborted or died shortly after birth. At this time Group 3, vaccine only, consisted of 16 animals, the original seven, and nine young. One sow in this group aborted about 12 weeks after the last injection of alcoholic precipitate. There was no evidence that this was due to infection. Group 4, untreated, had 19 animals, the original seven, and 12 young.

Post-mortem examination showed well marked splenic lesions in the sows of Groups 1 and 2. The spleens were nodular and hemorrhagic and varied in weight from 2 to 4 gm. *Br. abortus* was recovered from all. The agglutinin titres of the serum of these sows ranged from 1 : 250 to 1 : 1000. The males had no lesions and were negative serologically and on culture. The spleens weighed 0.8 and 0.9 gm. None of Group 1, vaccination and infection, was pregnant but two of the unvaccinated group exposed to infection were in early pregnancy. *Br. abortus* was not recovered from the placentas of these sows.

In this experiment, six intraperitoneal injections of alcoholic precipitate prior to breeding did not increase the breeding efficiency of guinea pigs that were subsequently exposed to infection. One of the control group that received only injections of precipitate aborted about 12 weeks after the last injection. The cause was not determined.

Summary

The specificity of alcoholic precipitate was shown *in vivo* by the fact that it was neutralized by injection of immune serum but was not affected by normal serum.

Immune serum absorbed with *Br. abortus* showed no protective action against alcoholic precipitate, the protective substance having been removed by absorption.

Alcoholic precipitate was as efficient as whole organisms in removing antibodies, as judged by the agglutination and complement fixation tests.

Fresh rabbit blood and serum caused a drop in temperature in guinea pigs treated by the intraperitoneal route. This serum was shown to be hemolytic for guinea-pig red cells *in vitro*.

Alcoholic precipitate of *Br. abortus* gave rise to agglutinins in the blood of guinea pigs and a rabbit. Where only one injection was given, agglutinin production was less common.

One injection of alcoholic precipitate in a rabbit produced very marked opsono-cytophagic activity against *Br. abortus* one week after injection and this persisted for the five weeks the animal was under test. The agglutinin titre was 1 : 25 one week after injection and disappeared the fourth week.

One-tenth gram of alcoholic precipitate by mouth did not produce any temperature reaction or ill effects in two guinea pigs that had been fasted overnight.

In the first immunity experiment four guinea pigs were given six intraperitoneal injections of 0.01 gm. of precipitate over a period of two weeks. These and two controls were exposed to infection by eye one week after the last injection. There was no indication of resistance to infection in these animals.

In the second experiment guinea pigs were given six intraperitoneal injections of alcoholic precipitate ranging from 0.0025 gm. to 0.02 gm. at five-day intervals. The last dose proved to be too large, causing a 60% mortality. The remaining animals and normal controls were exposed to infection by eye in three groups, one, two, and four weeks after the last injection of precipitate. All became infected as shown by agglutinin titres and spleen cultures but seven out of eight of the vaccinated animals had spleens which were normal in appearance and weight, whereas all the unvaccinated control spleens were distinctly nodular and those from the first group, which had been infected five weeks, were large. The difference in size was not as marked in those animals that had been held shorter periods.

In the third experiment, guinea pigs were given six intraperitoneal injections of alcoholic precipitate prior to breeding. Four groups consisting of six sows and one boar were used. The six normal sows had 12 healthy young. Six sows that were injected with alcoholic precipitate, but not exposed to infection, had nine young. There were no young in the vaccinated and unvaccinated groups that had been exposed to infection, but on post-mortem examination two of the latter group were found to be in early pregnancy. *Br. abortus* was not recovered from the placentas. One sow in each of the infected groups died following parturition. All showed well-marked spleen lesions which yielded *Br. abortus* on culture. There was no significant difference between the sows of these two groups. All showed lesions, were positive by the agglutination test and by cultural methods. Agglutinins disappeared from the blood of five of the animals that had been injected with alcoholic precipitate but not exposed to infection. One still showed a slight reaction at the conclusion of the experiment. One of the sows in this group aborted. There was no evidence that this was due to infection.

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A STUDY OF VARIOUS FRACTIONS OF *BRUCELLA ABORTUS*

IV. RESIDUES FROM WHOLE FILTRATE AND AFTER REMOVAL OF ALCOHOLIC PRECIPITATE[1]

BY RONALD GWATKIN[2]

Abstract

Residue obtained by evaporation of a filtered suspension of *Brucella abortus* after removal of the alcoholic precipitate, and residue from whole filtrate, were less toxic for guinea pigs than the alcoholic precipitate. Residue of the supernatant liquid had less complement binding power than the precipitate and produced only a weak skin reaction in infected guinea pigs. Residue of whole filtrate, prepared from five-day cultures, was as active as the precipitate as a complement binding antigen, but material prepared from two- and three-day growth was weaker. Injections of these substances failed to protect guinea pigs against infection by eye with *Brucella abortus*.

In previous papers (1, 2, 3) the writer reported on a study of an alcoholic precipitate obtained from a filtrate of a suspension of *Br. abortus*. This paper deals with the product obtained by evaporation of the supernatant fluid, after removal of the alcoholic precipitate, and also with the residue obtained by evaporation of an untreated filtrate. *Br. abortus* was grown on liver agar for several days, washed off with water or salt solution, shaken with glass beads, centrifuged and filtered through a Mandler candle, as described in the first article of this series (1).

Residue Obtained from Supernatant Fluid after Removal of Alcoholic Precipitate

Obviously the addition of alcohol to a filtrate of a shaken suspension of *Br. abortus* would not remove all the material present. It was therefore decided to obtain the residue in the supernatant fluid by evaporation. Through the kindness of Dr. A. D. Barbour a constant flow vacuum still was set up by means of which the fluid could be evaporated off at low temperature. The first lot was evaporated by moderate heat above a hot plate but this was discarded in favor of low-temperature evaporation.

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Experiment 1. Simple evaporation of supernatant fluid. Three hundred cc. of the supernatant fluid of Lot 7 alcoholic precipitate was filtered through a candle and evaporated to dryness above a hot plate and finished *in vacuo* over phosphorus pentoxide. The yield was greater than that of alcoholic precipitate, 0.45 gm. being obtained from 300 cc. The powder was markedly hygroscopic and went into solution very readily in water.

Intraperitoneal injection of residue. Two guinea pigs were injected by the intraperitoneal route as follows: No. 1 received 0.04 gm. and No. 2 was given 0.01 gm. of residue. Temperatures are shown in Table I.

TABLE I

TEMPERATURES OF GUINEA PIGS INJECTED WITH RESIDUE

	11:00 a.m.	1:30 p.m.	3:30 p.m.	5:00 p.m.	6:30 p.m.	9:00 a.m.	12:00 m.
No. 1	103.0	102.6	103.0	102.6	105.0	103.0	103.2
No. 2	103.0	102.4	101.6	101.2	102.4	102.2	102.6

It will be seen that the residue did not cause a drop in temperature of any extent. There was a passing rise in No. 1. The heart action was affected for a few hours but the animals were not otherwise affected.

Complement fixation test on precipitate and residue. A suspension of 0.01 gm of each in 2.0 cc. saline was made and diluted 1 : 25, 1 : 100 and 1 : 500 The results of the test of residue compared with alcoholic precipitate from the same filtrate are given in Table II.

TABLE II

COMPLEMENT FIXATION TEST ON RESIDUE AND PRECIPITATE OF LOT 7

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
Alcohol precipitate	1 : 25	4	4	2	2	0	0	0
Residue	1 : 25	4	4	4	4	4	3	0
Al. ppt.	1 : 100	4	4	4	4	0	0	0
Residue	1 : 100	1	1	1	0	0	0	0
Al. ppt.	1 : 500	4	4	4	4	4	0	0
Residue	1 : 500	0	0	0	0	0	0	0

4 = complete fixation. 3 = 75% fixation. 2 = 50% fixation. 1 = 25% fixation. 0 = no fixation.

The residue 1 : 25 was slightly higher than the 1 : 500 precipitate, but residue 1 : 100 showed only a trace of fixation and 1 : 500 gave none. The alcoholic precipitate was a much stronger antigen, but, as in all our tests, seemed to be inhibited in the lower dilutions.

Experiment 2. Lot 2 supernatant residue. The supernatant fluid from several lots was filtered through fine paper and run through the vacuum still. The temperature of the bath containing the distilling flask was kept at about 50° C. The fluid was reduced to 40 cc. and finished over a water bath between 70 and 80° C. and then *in vacuo* over phosphorus pentoxide. A yield of 8.3 gm. was obtained. Temperatures of injected guinea pigs went lower with this lot than with the first one, probably owing to larger dosage, but not as low as those of the precipitate animals. It proved to be fatal in doses of 0.1 cc. as four out of six injected guinea pigs died.

Protection test with residue. Six guinea pigs were given an intraperitoneal injection of 0.04 gm. This was the maximum injection given with the previous lot. It caused no ill effects and little change in temperature. The second injection of 0.08 gm. five days later caused more drop in temperature but was otherwise well tolerated. The third injection, of 0.1 gm., at a similar interval did not produce much fall in temperature, but two animals died from peritonitis. The fourth, fifth and sixth injections were the same. After the conclusion of injections there were only three animals left. These and three controls were exposed to infection by eye with one drop of a suspension of *Br. abortus* equal to Gates No. 1 two weeks after the last injection. Another vaccinated animal died two weeks after exposure to infection. The spleen appeared normal and on culture was negative.

Agglutination tests were made weekly following exposure to infection and results are shown in Table III.

TABLE III

AGGLUTINATION TESTS ON GUINEA PIGS IN RESIDUE PROTECTION EXPERIMENT

		Infected						
		May 26	June 26	July 3	July 10	July 17	July 24	July 31
Vaccinated	2	—	1 : 25	1 : 25	1 : 25	Dead		
	4	—	1 : 100	1 : 100	1 : 100	1 : 100	1 : 250	1 : 500
	6	—	1 : 50	1 : 25	—	1 : 100	1 : 100	1 : 500
Controls	1			—	—	1 : 100	1 : 250	1 : 500
	2			—	—	1 : 100	1 : 250	1 : 500
	3			—	—	1 : 100	1 : 250	1 : 500

These animals were killed four weeks after exposure to infection. The spleens of all were slightly enlarged but no difference was observable between vaccinated animals and controls. *Br. abortus* was recovered from all. There was no evidence that any protection had developed from injections of this residue.

Intradermal test with residue. A suspension of 0.05 gm. of residue in 10 cc. of water was prepared. Half of this was treated by the addition of 0.25% formalin. Both were left in the refrigerator for two days. Normal

and infected guinea pigs were given intradermal injections of 0.1 cc. of formolized and untreated suspension. There was no reaction from either product in the negative animal but both produced a slight reaction in the infected guinea pig. It was not nearly as clear as that produced by the precipitate.

Experiment 3. Lot 3 supernatant residue. This was obtained from the supernatant fluids containing 2.5, 5 and 10 volumes of alcohol which were used to prepare precipitates in a previous experiment. It was prepared as in Experiment 2. Varying the amounts of alcohol did not appear to affect the residue. All three were about the same in complement-binding activity. The 5-volume precipitate and residue only need be given to illustrate the difference between the precipitate and residue. This is shown in Table IV.

TABLE IV

COMPLEMENT FIXATION TEST WITH 5-VOLUME PRODUCTS OF LOT 8

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500
Precipitate	1 : 25	Anticomplementary				
Residue	1 : 25	1	0	0	0	0
Precipitate	1 : 100	4	4	4	0	0
Residue	1 : 100	0	0	0	0	0
Precipitate	1 : 500	4	4	4	4	0
Residue	1 : 500	0	0	0	0	0

This residue gave only slight fixation in the first tube of the lowest dilution as compared with 1 : 250 in the 1 : 500 dilution of precipitate.

Guinea pigs were injected with 0.06 gm. of each residue. There was a rise of temperature in one animal and a very slight drop in the other two. All remained well. On the other hand three guinea pigs injected with 0.02 gm. of the precipitate at 2:00 p.m. were dead the following morning.

Evaporated Whole Filtrate

The alcoholic precipitate and its supernatant fluid residue having been tried, it was decided to obtain the solids in a filtrate with as little heating as possible. The bacteria were grown and handled by the usual method of producing alcoholic precipitate as far as the filtrate stage except that the suspension was not heated.

Experiment 4. Lot 1 evaporated whole filtrate (heated). Br. abortus was grown for five days on liver agar. It was washed off with salt solution, shaken for 24 hr., centrifuged and filtered through paper and a Mandler candle. The filtrate was evaporated down to about 20 cc. in the vacuum still. It was then dialyzed 48 hr. in running tap water, evaporated to dryness over a water bath between 70 and 80° C., and finished *in vacuo* over phosphorus pentoxide.

Intraperitoneal injection of guinea pigs. Two guinea pigs were given intraperitoneal injections of 0.02 gm. in 2.0 cc. water. Temperatures are shown in Table V.

TABLE V

INTRAPERITONEAL INJECTION OF EVAPORATED WHOLE FILTRATE

	9:15 a.m.	11:15 a.m.	2:00 p.m.	4:00 p.m.	6:00 p.m.	9:00 a.m.	11:45 a.m.	2:00 p.m.
No. 1	103.2	100.8	97.4	96.0	95.0	99.4	101.8	103.0
No. 2	103.6	101.0	96.8	97.2	97.2	Killed		

These animals showed the same symptoms as were produced by alcoholic precipitate. No. 1 recovered, No. 2 was killed as the rectum was everted. As would be expected, the temperatures did not go as low as with precipitate because less than 0.01 of the alcohol precipitable substance would be present in this injection.

Complement fixation test. The evaporated whole filtrate gave exactly the same results as the alcoholic precipitate prepared from the same filtrate. This was somewhat surprising in view of the difference in complement-binding value between precipitate and supernatant residue. The results from the evaporated whole filtrate are given in Table VI. The precipitate readings were identical.

TABLE VI

COMPLEMENT FIXATION TEST OF EVAPORATED WHOLE FILTRATE

	1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
1 : 25	4	4	4	3	0	0	0
1 : 100	4	4	4	4	0	0	0
1 : 500	4	4	4	4	4	1	0

This substance showed the same tendency to inhibition in the lower dilutions that was manifested by the precipitate which was prepared from a portion of the same filtrate.

Experiment 5. Lot 1 evaporated whole filtrate (unheated). This lot differed from that in Experiment 4 in the fact that the suspension was not heated above 45° C. Forty-eight-hour growth of *Br. abortus* was washed off with water, shaken 22 hr. with glass beads, centrifuged and filtered through a fine candle. The clear filtrate was evaporated in the vacuum still with the water bath at about 45° C. It was not dialyzed. The last portion of the residual fluid was dried in the incubator at 37° C. and then over phosphorus pentoxide *in vacuo*. The finished product was very hygroscopic.

Intraperitoneal injection of evaporated filtrate. One guinea pig was given an intraperitoneal injection of 0.01 gm. in 2.0 cc. water and another was given 0.04 gm. Temperatures of these animals are shown in Table VII.

TABLE VII

INTRAPERITONEAL INJECTION OF EVAPORATED FILTRATE

	10:00 a.m.	12:00 m.	2:00 p.m.	4:00 p.m.	9:00 a.m.
No. 1 0.01 gm.	102.2	103.6	103.4	102.8	102.0
No. 2 0.04 gm.	102.8	98.0	95.0	94.0	Dead

The smaller injection produced a slight rise in temperature while the larger dose acted like alcoholic precipitate. It caused a rapid fall in temperature, and death.

Complement fixation test with evaporated filtrate. A suspension of 0.01 gm. in 2.0 cc. saline was diluted 1 : 25, 1 : 100 and 1 : 500.

These dilutions were set up in the ordinary way with a positive serum. The regular antigen was also set up as a check on this material. The results are shown in Table VIII.

TABLE VIII

COMPLEMENT FIXATION RESULTS WITH EVAPORATED WHOLE FILTRATE

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
Lot 1 unheated	1 : 25	4	4	4	4	4	0	0
	1 : 100	0	0	0	0	0	0	0
	1 : 500	0	0	0	0	0	0	0
Regular antigen	1 : 15	4	4	4	4	4	4	0

The complement-binding power of this material was not nearly as great as that of the heated material in Experiment 4. This lot was only grown for two days as compared with five in the case of Experiment 4, which may be responsible for the difference.

Experiment 6. Lot 2 evaporated whole filtrate. Br. abortus was grown for three days on the usual liver agar. The growth was washed off with water and shaken for 24 hr. with glass beads. It was not heated. The suspension was centrifuged and filtered through a fine Mandler candle. A pane of glass was supported over a hot plate in such a manner that it merely became warm and not hot. A current of air was blown across the plate by means of a fan. The filtrate was poured on to cover the surface in a thin layer. It was dry in about two hours, when it was scraped off and the drying completed in a

desiccator under negative pressure over phosphorus pentoxide. It was ground up and tightly sealed in a vial. This material was very hygroscopic and became sticky during the short time required to weigh it. During the whole process it was not exposed to heat.

Complement fixation reaction. A suspension of 0.01 gm. in 2.0 cc. water was prepared. This was diluted to 1 : 25, 1 : 100 and 1 : 500 and set up with a positive serum and also the regular antigen. Results are given in Table IX.

TABLE IX

COMPLEMENT FIXATION TEST ON LOT 2 EVAPORATED WHOLE FILTRATE (UNHEATED)

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500	1 : 5000
Lot 2	1 : 25	4	4	4	4	4	4	4	0
	1 : 100	4	4	4	4	4	4	0	0
	1 : 500	4	0	0	0	0	0	0	0
Regular antigen	1 : 15	4	4	4	4	4	4	4	0

This lot of material differed from the first lot in that it did not show the tendency to inhibition in the lower dilutions and was not as strong an antigen. In these respects it resembled Lot 1 unheated (Exp. 5), but was active in a higher dilution than that product. Cultures from which the suspensions were prepared had been incubated one day longer.

Protection test with Lot 2 evaporated whole filtrate (unheated). Six guinea pigs were given intraperitoneal injections of this material, each receiving 0.01 gm. in 2 cc. water. No. 3 died after the third injection and showed the usual changes of inflammation of the peritoneum and fibrinous exudate on the liver. The others received six injections.

These animals were bled and exposed to infection with a suspension of *Br. abortus* equal to 1 cc. on the Gates nephelometer, two weeks after the last injection. There was no evidence of protection and *Br. abortus* was recovered from the spleens of these animals.

Precipitation of Filtrate with Ammonium Sulphate

Ammonium sulphate was tried in place of alcohol for precipitation of the filtrate of a suspension of *Br. abortus*. Only one experiment was carried out as it was considered unwise to change the methods of precipitating until the alcoholic precipitate had been more fully worked out.

Experiment 7. Ammonium sulphate precipitate. Br. abortus was grown for five days on liver agar. It was washed off with salt solution, heated, and shaken for 24 hr. It was then passed through a Sharples supercentrifuge and a fine Mandler candle. One portion of the clear filtrate was treated in the usual way with five volumes of 95% alcohol. To another lot of 200 cc. was

added 160 gm. of ammonium sulphate, C.P. Next morning the ammonium sulphate precipitate had collected on the surface of the fluid. It was removed, dialyzed, dried and ground. Guinea pig injections and complement fixation tests were carried out with both precipitates. Results of the complement fixation test are shown in Table X. The usual dilutions of antigen were employed.

TABLE X

COMPLEMENT FIXATION TEST WITH AMMONIUM SULPHATE AND ALCOHOL PRECIPITATES

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000
Ammonium sulphate	1 : 25	4	4	4	4	0	0
Alcohol	1 : 25	4	4	4	3	0	0
Ammonium sulphate	1 : 100	4	4	4	4	2	0
Alcohol	1 : 100	4	4	4	4	0	0
Ammonium sulphate	1 : 500	4	4	4	4	0	0
Alcohol	1 : 500	4	4	4	4	4	1

The ammonium sulphate precipitate showed a little more fixation in the lower dilutions but in the 1 : 500 dilution of the suspension of these products (actually 1 : 100,000 dilution of the precipitates) the alcoholic precipitate was more antigenic.

Intraperitoneal injection of guinea pigs with both precipitates. Two guinea pigs injected with 0.02 gm. of the precipitates in 2.0 cc. water showed a similar fall in temperature and died the next day. Two more guinea pigs were injected with 0.02 gm. of alcoholic precipitate and two with the same dose of the ammonium sulphate product. The temperature drop was very similar in all four animals. One of the alcoholic-precipitate guinea pigs died and the other three animals recovered. It appeared from this that the material precipitated with ammonium sulphate was very similar to the alcoholic product.

Summary

The residue in the supernatant fluid after precipitation by alcohol was obtained by evaporation *in vacuo* at about 50° C., final drying at 70–80° C. for a short time over a water bath and then *in vacuo* over phosphorus pentoxide.

The residue was low in complement fixing power and was not as toxic for guinea pigs in equal dosage. Injections of 0.1 gm. proved fatal to these animals. Intradermal injections in infected guinea pigs produced only a slight reaction.

Two guinea pigs that had been given six injections of residue at five-day intervals and exposed to infection by eye two weeks after the last injection showed no evidence of protection. They were shown, by post-mortem and cultural examination, to be infected.

Filtrates of suspensions of heated and unheated *Br. abortus* were evaporated to dryness by vacuum distillation and low temperatures. The first lot prepared from a five-day growth was more efficient as a complement-fixing antigen than either of the other two lots, which had been grown for two and three days only, in spite of the fact that Lot 1 had been heated. The three-day growth product was stronger than that prepared from the two-day cultures. Intraperitoneal injections caused a fall in temperature in injected guinea pigs. Larger doses were required to kill than in the case of the precipitate. Intraperitoneal injections of this material did not protect guinea pigs against infection by eye. A precipitate produced with ammonium sulphate appeared to be the same as the alcoholic product as far as it was studied.

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A STUDY OF VARIOUS FRACTIONS OF *BRUCELLA ABORTUS*

V. ALCOHOLIC PRECIPITATES PREPARED FROM A DISSOCIATED STRAIN OF *BR. ABORTUS* AND FROM *E. COLI* AND *B. SUBTILIS*[1]

BY RONALD GWATKIN[2]

Abstract

An alcoholic precipitate from an R strain of *Brucella abortus* resembled the organism from which it was obtained. Toxicity was low, it produced only a slight reaction in the skin of an infected guinea pig and it had no antigenic power in the complement fixation test. An alcoholic precipitate of *E. coli* was more toxic than any obtained from *Br. abortus*. The effects of intraperitoneal injections of colon precipitate were modified by anti-colon serum. Intraperitoneal injection of an alcoholic precipitate of *B. subtilis* produced no change in guinea pigs other than a slight fall in temperature.

This article is a continuation of the work previously published on alcoholic precipitates and other portions of filtrates of *Br. abortus* (1–4). The experiments with *E. coli* and *B. subtilis* were introduced in order to see whether the toxicity found in the *Brucella* preparations would also occur in commensals and saprophytes. *E. coli*, which at times may be pathogenic, proved to be more toxic than any of the *Brucella* strains examined, but the toxicity of each was specific.

Experiment 1. Alcoholic precipitate of a dissociated strain of Br. abortus. An alcoholic precipitate was prepared from a dissociated strain of *Br. abortus*, the antigenic qualities of which have previously been reported (5, 6). The procedure was the same as that described for the regular strains (1). Centrifugation was simplified as the bacteria went out of suspension overnight.

Intraperitoneal injections. Three guinea pigs weighing about 350 gm. were given intraperitoneal injections of 0.01, 0.02 and 0.04 gm. of alcoholic precipitate. Temperatures of these animals are given in Table I.

TABLE I

TEMPERATURES OF GUINEA PIGS INJECTED WITH R ALCOHOLIC PRECIPITATE

	10:00 a.m.	12:00 m.	2:00 p.m.	5:00 p.m.	9:00 a.m.
No. 1 0.01 gm.	102.2	98.4	97.0	98.0	102.6
No. 2 0.02 gm.	*102.0*	*101.2*	*100.0*	*98.2*	*102.4*
No. 3 0.04 gm.	102.8	96.0	95.4	96.2	101.8

These animals showed symptoms the same as those produced by injections of S precipitate, but recovery was rapid and temperatures also returned quickly to normal in comparison with those of guinea pigs injected with the regular precipitate.

[1] *Manuscript received September 27, 1934.*
Contribution from the Department of Pathology and Bacteriology, Ontario Research Foundation, Toronto, Canada.

[2] *Research Fellow.*

Intradermal test with R alcoholic precipitate. A normal and an infected guinea pig were injected intradermally with 0.1 cc. of a 1 : 250 suspension of precipitate. There was no reaction in either animal at 24 hr. but the following day there was a slight reaction in the infected guinea pig, which was not equal to that produced by the regular precipitate.

Complement fixation test. A suspension of 0.01 gm. of R precipitate in 2 cc. saline was prepared. This was diluted 1 : 25, 1 : 100 and 1 : 500, making dilutions of the precipitate of 1 : 5000, 1 : 20,000 and 1 : 100,000. These were the dilutions used for the regular precipitate. Results are shown in Table II.

TABLE II

COMPLEMENT FIXATION TEST WITH R ALCOHOLIC PRECIPITATE

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
	1 : 25	0	0	0	0	0	0	0
	1 : 100	0	0	0	0	0	0	0
	1 : 500	0	0	0	0	0	0	0
Lot 6	1 : 500	4	4	4	4	2	0	0
Reg. antigen	1 : 15	4	4	4	4	4	4	0

A positive serum with complement binding titre of 1 : 1000 did not produce any fixation with R precipitate in the same dilutions in which S precipitate and the regular antigen showed definite fixation of complement.

The results from R alcoholic precipitate coincide with the behavior of suspensions of the R organism. In the work to which reference has been made, R had no antigenic value in the complement fixation test. It was harmless for guinea pigs until after a series of animal passages but it did produce a skin reaction in infected guinea pigs. No further work was carried on with R precipitate. Earlier work with this organism itself had shown that living cultures had no immunizing value against subsequent exposure to infection with the S prototype.

Experiment 2. Alcoholic precipitate of E. coli. An alcoholic precipitate was prepared from a strain of colon isolated from feces. The preparation was as already described. The precipitate appeared to be more soluble than that prepared from *Br. abortus* and there was no difficulty in getting it into solution.

Intraperitoneal injection of guinea pigs with colon precipitate. Two guinea pigs were given intraperitoneal injections of colon precipitate. No. 1 received 0.01 gm. and No. 2 was given 0.04 gm. in 2 cc. water. Death occurred as rapidly, and, in the larger dose, more rapidly than following *Brucella* precipitate. Temperatures are shown in Table III. Symptoms were the same as those produced by *Brucella* precipitate.

TABLE III

TEMPERATURES OF GUINEA PIGS INJECTED WITH COLON PRECIPITATE

	10:00 a.m.	12:15 p.m.	2:00 p.m.	4:00 p.m.	9:00 a.m.
No. 1	102.6	94.0	94.0	94.0	Dead
No. 2	102.8	94.0	94.0	Dead	—

It will be seen that the temperature of the injected animals dropped very rapidly, even in the case of the smaller dose, and death of the animal receiving the larger dose occurred five hours after injection.

Effect of anti-abortus serum on injections of colon precipitate. In view of the toxicity of this substance and to make sure that the action of alcoholic precipitate was not merely a general one, common to all organisms and without specificity, guinea pigs were given intraperitoneal injections of this material and normal and immune serum. The latter was rabbit serum which had proved so effective in a previous experiment (3). Temperatures of these animals are shown in Table IV. No. 1 received 5.0 cc. immune serum and 0.02 gm. of precipitate while No. 2 was given 5.0 cc. negative serum and 0.02 gm. precipitate.

TABLE IV

TEMPERATURES OF GUINEA PIGS INJECTED WITH NORMAL AND ANTI-ABORTUS SERUM AND COLON ALCOHOLIC PRECIPITATE

	11:00 a.m.	12:30 p.m.	2:00 p.m.	3:30 p.m.	5:00 p.m.	9:00 a.m.
No. 1	102.8	94.0	94.0	94.0	94.0	Dead
No. 2	102.8	97.0	94.0	94.0	94.0	Dead

In 1½ hours the temperature of the animal that received anti-abortus serum and precipitate had dropped to 94. On the next reading the normal-serum animal was also 94. Both were dead the following morning. No anti-colon serum was available at that time to try the effect of a specific anti-serum on colon precipitate, but the experiment showed that anti-abortus serum capable of protecting guinea pigs against *Brucella* precipitate had no effect against colon precipitate.

Experiment 3. Effect of anti-colon serum on colon alcoholic precipitate. A rabbit was given three injections of phenolized suspension of *E. coli* by the subcutaneous route, followed by an injection of living culture. Three weeks after the last injection, blood was drawn from the heart, the serum was collected and was heated at 56° C. for 30 min. An alcoholic precipitate had been prepared from the same strain. The serum had an agglutinin titre of only 1 : 100. Blood was collected from a normal rabbit, and when this was

examined later for agglutinins against *E. coli* it was found that partial agglutination occurred in 1 : 10, 1 : 25 and 1 : 50, which would account for the protection afforded by this supposedly negative serum. Three guinea pigs were injected by the intraperitoneal route as follows:

1. 0.01 gm. alcoholic ppt. + 6 cc. water
2. 0.01 gm. alcoholic ppt. + 6 cc. normal (?)serum.
3. 0.01 gm. alcoholic ppt. + 6 cc. anti-colon serum.

Temperatures of these guinea pigs are shown in Table V.

TABLE V

TEMPERATURES OF GUINEA PIGS

	10:00 a.m.	12:00 m.	2:00 p.m.	4:00 p.m.	5:00 p.m.	9:00 a.m.	11:00 a.m.	12:00 m.	9:00 a.m.
No. 1	102.0	93.0	93.0	93.0	93.0	93.0	93.0	93.0	Dead
No. 2	101.2	97.0	97.0	97.4	98.0	98.8	100.0	101.0	102.0
No. 3	101.4	100.2	100.8	97.2	96.0	99.0	101.8	102.0	102.2

The temperature of the guinea pig that received precipitate alone dropped very rapidly and the animal died. The temperature of the anti-colon-serum guinea pig came down more slowly and went up more rapidly than that of the animal which received the serum from an uninjected rabbit. This serum, however, contained agglutinins against *E. coli*, and both serum-treated rabbits recovered.

Experiment 4. Alcoholic precipitate of B. subtilis. This organism was grown on liver agar and an alcoholic precipitate was prepared. *B. subtilis* was chosen as being one which could not be considered to be pathogenic at any time, whereas the writer has had many virulent strains of *E. coli* from animals. The precipitate was similar in appearance to *Brucella* and colon preparations. Two guinea pigs were given intraperitoneal injections of the precipitate. No. 1 received 0.01 gm. in 2.0 cc. water and No. 2 was given 0.04 gm. in 2 cc. water. Temperatures of these animals are given in Table VI.

TABLE VI

TEMPERATURES OF GUINEA PIGS INJECTED WITH *B. subtilis* PRECIPITATE

	9:30 a.m.	11:15 a.m.	2:30 p.m.	4:00 p.m.	9:15 a.m.	11:00 a.m.	2:30 p.m.	4:00 p.m.	9:00 a.m.
No. 1	103.0	103.0	101.0	99.4	102.6	101.6	102.0	100.6	102.2
No. 2	*103.1*	*101.2*	*102.8*	*102.6*	*100.0*	*101.2*	101.2	100.4	101.4

There was some drop in temperature in these guinea pigs but they appeared well throughout the period of observation, and there was not the illness associated with *Brucella* and colon precipitate.

Summary

Intraperitoneal injections of R alcoholic precipitate produced symptoms similar to those following injections of S precipitate. They were less marked and the temperature returned more rapidly to normal. A slight skin reaction was produced by R precipitate in an infected guinea pig. This was not as well marked as the S-precipitate reaction although the suspension of R was stronger. The R precipitate did not react as an antigen in the complement fixation test.

An alcoholic precipitate of *E. coli* produced a more violent reaction in guinea pigs than the *Brucella* precipitate. The effect of intraperitoneal injections of colon precipitate was not modified by anti-abortus serum. Guinea pigs were protected by serums containing colon antibodies.

An alcoholic precipitate of *B. subtilis* produced some fall in temperature following the intraperitoneal injection, but no other disturbance was noticed in the injected guinea pigs.

Acknowledgments

The writer is grateful to T. Lloyd Jones, B.V.Sc., for his assistance and to Dr. H. B. Speakman and Dr. S. Hadwen for advice and support in connection with this work, which was made possible by a grant from the Department of Agriculture of Ontario.

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A STUDY OF VARIOUS FRACTIONS OF *BRUCELLA ABORTUS*

VI. EXTRACT OF DRIED AND GROUND ORGANISMS[1]

By Ronald Gwatkin[2]

Abstract

Filtrates prepared from dry, ground *Brucella abortus* (Huddleson) caused a fall in temperature and death in some cases in guinea pigs injected by the intraperitoneal route. The filtrates were not as active as bacterial suspension antigen in the complement fixation test. They produced reactions in the skin of infected guinea pigs. Intraperitoneal injections failed to protect guinea pigs against infection by eye with *Br. abortus*. The preparation of this material gave rise to marked symptoms in a hypersensitive human subject.

Introduction

In previous papers the writer has recorded experiments with alcoholic precipitate and various residue antigens (1–5). This article deals with a filtered extract of dried and ground *Br. abortus*.

This method was explained to the writer by Dr. I. Forest Huddleson of Michigan. Dr. Huddleson found that this extract was toxic for guinea pigs but it did not apparently have any immunizing value.

Flat bottles of liver agar, pH 6.6, were seeded with an aerobic culture of *Br. abortus* of recent isolation. After three days at 37° C., the growth was washed off with saline, centrifuged, and the sediment dried for a day in the incubator and then over phosphorus pentoxide *in vacuo*. The dried material was ground for 24 hr. in a ball mill with ⅜-in. steel balls. Salt solution containing 1% toluene and 1% ether was then added and the mixture was rotated for a couple of hours. It was then incubated at 37° C. for 48 hr. The next step was filtration through paper treated with fuller's earth, after which it was heated for 10 min. at 100° C. and concentrated by evaporation with a fan and a current of warm air. It was then filtered through a fine Mandler candle.

Guinea pig inoculation. One guinea pig was given an intraperitoneal injection of 5.0 cc., while a second received 1.0 cc. by the same route. Both animals twitched for a few minutes after injection and then settled down quietly. The first animal became very ill within a few hours and its temperature dropped from 102.8 to 98.0° F. The following day it was 94.0 (the lowest our thermometer would record) and it remained there until the following day when it rose to 101.4. The temperature of the other guinea pig did not go below 97.6. Both recovered.

[1] *Manuscript received September 27, 1934.*
Contribution from the Department of Pathology and Bacteriology, Ontario Research Foundation, Toronto, Canada.
[2] *Research Fellow.*

Four more guinea pigs were injected with doses of from 5.0 cc. to 0.01 cc. by the intra-abdominal route at 2:30 p.m. Table I gives injections and temperature reactions of these animals.

TABLE I

GUINEA PIGS INJECTED WITH GROUND EXTRACT FILTRATE

No.	Injection, cc.	Temperatures			
		2:30 p.m.	3:30 p.m.	5:30 p.m.	9:00 a.m.
1	5.0	102.8	97.2	94.0	Dead
2	1.0	102.4	101.0	101.2	101.8
3	0.1	102.4	104.2	103.0	103.0
4	0.01	102.6	102.4	103.4	101.8

No. 1 died during the night. There was fluid in the abdominal cavity, fibrinous exudate on liver, inflammation of peritoneum and intestines. Cultures were negative.

In order to determine what effect might be produced by the medium itself, 5.0 cc. of liver agar was melted and 10.0 cc. saline added to keep it liquid. An injection of 5.0 cc. of this produced a drop in temperature of only 1.5°F.

The five surviving guinea pigs from the above series and three normal guinea pigs were exposed to infection by eye with one drop of a suspension of *Br. abortus* equal in density to tube No. 2 of McFarland's nephelometer, three weeks after they had received the injections of filtrate. Agglutination tests were carried out weekly and the animals were killed 42 days after exposure to infection. There was no suggestion of any protection in the injected animals, all being shown to be infected.

Ground extract filtrate II. Another filtrate was prepared in the same manner as the first one, except that chloroform in excess was added during the period of extraction in place of toluene, and the filtrate was not concentrated by evaporation. Two guinea pigs injected with 5.0 cc. and 1.0 cc. showed a drop in temperature but survived.

Ground extract filtrate III. A third filtrate produced death in a guinea pig injected with 5.0 cc. The animal lived two days and there was a large amount of sterile pus in the abdominal cavity. This filtrate consisted of 10 gm. of dry powder in 250 cc. saline and was produced in the same manner as filtrate No. 1.

Complement fixation tests with Lots 1, 2 and 3. Filtrate No. 1 was diluted 1 : 25 and 1 : 1000. Lots 2 and 3 were diluted 1 : 10 and 1 : 100 and all were used as antigens in the complement fixation test with a positive serum. The tests were carefully controlled with antigen and serum tubes, details of which need not be shown here. Table II gives the results with these and our regular *Br. abortus* antigen.

TABLE II

COMPLEMENT FIXATION TEST ON LOTS 1, 2 AND 3

		1 : 25	1 : 50	1 : 100	1 : 250	1 : 500	1 : 1000	1 : 2500
Lot 1	1 : 25	4	4	0	0	0	0	0
	1 : 1000	4	4	4	2	0	0	0
Lot 2	1 : 10	4	4	1	0	0	0	0
	1 : 100	4	4	4	0	0	0	0
Lot 3	1 : 10	4	4	4	2	0	0	0
	1 : 100	4	4	4	0	0	0	0
Regular antigen		4	4	4	4	4	4	0

4 = Complete fixation. 3 = 75% fixation. 2 = 50% fixation. 1 = 25% fixation. 0 = No fixation.

It will be noted that the filtrates were not as strong antigens as the regular suspension of *Br. abortus*. This was a bacterial suspension of the organism of the same density as the agglutination antigen (No. 1, McFarland's nephelometer) diluted 1 : 15 for use in the complement fixation test. In Lot 1 the 1 : 1000 dilution showed more fixation than 1 : 25. The same was seen with Lot 2 in dilutions of 1 : 10 and 1 : 100. Lot 1 was repeated with the same result in 1 : 25 and a 1+ instead of a 2+ in the last tube of the 1 : 1000 dilution, which is within the range of experimental error. The same inhibition had been observed with the various alcoholic precipitates.

Skin tests. Infected and normal guinea pigs were given intradermal injections of 0.1 cc. of two of the filtrates. Lot 2 gave some reaction in both infected and normal animals but that in the latter disappeared after two days, while a small, hard swelling persisted in the infected one. Lot 3 gave a well marked swelling in the infected pig but produced no reaction in the normal animal. The preparation of this material produced the same symptoms in a hypersusceptible human subject as were previously recorded for the alcoholic precipitate (1).

Immunity experiment. Four guinea pigs were given six intraperitoneal injections of filtrate No. 3 over a period of two weeks. The first five injections were 2.0 cc. each and the sixth was 4.0 cc. One week after the last injection these animals and two normal guinea pigs were exposed to infection by eye with one drop of *Br. abortus* suspension equal in density to tube No. 2 of McFarland's nephelometer. The only pregnant female in the vaccinated group aborted 17 days after infection. These animals were killed a month after exposure, weekly agglutination tests having shown that all were infected. Post-mortem examination showed enlargement of the spleen. Cultures were positive. There was no delay in agglutinin production in the vaccinated pigs, nor any other indication that the injections of filtrate had done anything to delay the onset of infection.

Summary

A filtrate was prepared from dry, ground *Br. abortus* as suggested by Dr. I. Forest Huddleson. Intraperitoneal injections caused a fall of temperature in guinea pigs and, in some cases, death. The filtrates were not as strong complement-binding antigens as a bacterial suspension. There was a tendency to inhibition of fixing power in the lower dilutions. The filtrates produced skin reactions when injected into infected guinea pigs and, in one case, in a normal animal also. Six injections of filtrate over a period of two weeks failed to protect guinea pigs against subsequent infection by eye.

The preparation of this material gave rise to symptoms simulating undulant fever in a hypersensitive human subject.

Acknowledgments

The writer is grateful to Dr. I. Forest Huddleson for his interest in the problem and to Dr. H. B. Speakman and Dr. S. Hadwen for advice and support throughout this work, which was made possible by a grant from the Department of Agriculture of Ontario.

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SOME OBSERVATIONS ON THE ALLERGY TO TUBERCULIN OF GUINEA PIGS VACCINATED WITH B.C.G.[1]

By Armand Frappier[2] and Victorien Fredette[3]

Abstract

Experiments indicate that the development of allergy in guinea pigs vaccinated with B.C.G. (0.0025, 0.005 or 0.010 gm.), is relatively slow and varies with individuals. It is possible to measure the degree of skin sensitivity by serial intradermal tests with decreasing doses of tuberculin. Classification as to degree of allergy and study of the development of hypersensitivity can hardly be effected on the basis of strong doses of tuberculin. In fact, early positive reaction is not always a sign of lasting hypersensitivity and many guinea pigs, which respond at the same time to a strong dose of tuberculin, can be differentiated as to degree of hypersensitivity by tests with smaller doses of tuberculin. Most of the subcutaneously vaccinated guinea pigs developed allergy in from five to twenty days. The maximum allergy was reached in from one to two months. In a large number of the more sensitive animals the dose was then reduced to 0.00001 cc. and the reaction was maintained during the third and fourth months and perhaps longer. A few animals were sensitive to 0.000005 cc. in the fourth month.

The observation has been verified that in subcutaneously vaccinated guinea pigs there is, as a rule, no correlation between small variations in the size of the dose of B.C.G. and the degree of allergy produced. Nevertheless, of the animals vaccinated intraperitoneally, those inoculated with the larger quantities of bacilli were more highly sensitized. This distinction was made by means of the smaller doses of tuberculin.

Introduction

Most authors appear to agree as to the innocuousness of B.C.G. They apparently consider the attenuation of this virus to be constant and irreversible. They also admit that B.C.G., introduced into the organism, gives rise to a certain "premunition" against virulent tuberculous infections, and a more or less pronounced allergic or hypersensitive state. But a battle wages around the quantitative appreciation of the value and length of the immunity and hypersensitivity thus conferred.

There is no doubt that the problems of tuberculous infection in general will be more easily solved with the advent of more precise understanding of the effects of immunity and hypersensitivity on its evolution. This is the actual reason why most workers turn to the exploration of compared limits of allergy and immunity in tuberculosis.

Following Romer (24), Hamburger (16), Debré *et. al.* (14, 15), Paraf (19), Bezancon and de Serbonnes (1), and others, many authors, *e.g.*, Boquet *et. al.* (7–13), Saenz (28, 29), Birkhaug (2), and Petroff (21–23), have undertaken to study the ante-allergic and allergic periods in animals inoculated by different modes, with different doses of dead or living tubercle bacilli of varied virulence, or extracts of tubercle bacilli. The test dose was generally of strong concentration.

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[2] *Director of the Departments of Bacteriology, University of Montreal, and St. Luke's Hospital, Montreal.*

[3] *Bacteriologist, University of Montreal.*

Although the earliest and more lasting reactions to strong doses of tuberculin are often characteristic of the most sensitive animals, these cannot serve either to measure effectively the degree of hypersensitivity or to follow its progressive development in any animal.

Long ago Koch (17, 18) noted that, in infected animals, the lethal dose of tuberculin decreases with the elapsed time since infection. Borrel (3), confirmed this finding. In 1929 Parish and Okell (20) reported their observations on the development of hypersensitivity to intradermal doses of tuberculin. They found that in guinea pigs infected with a large quantity of virulent bacilli (0.5 mg.) the doses decreased with time from 0.004 to 0.00005 cc.

The work thus begun appears to have been neglected by the majority of workers who failed to measure the degree and development of allergy by means of serial and intradermal reactions to decreasing doses of tuberculin. Should the results obtained with this procedure prove to be reliable it would be interesting to study the developments of immunity in the light of the allergy behavior thus determined.

The work of determining, by serial and intradermal reactions effected with decreasing doses of tuberculin, the development of hypersensitivity in our guinea pigs inoculated with B.C.G., has been undertaken. This preliminary research will serve in a proposed study of the relations between the states of allergy and immunity in these vaccinated animals.

This work was already under way when Boquet and Bretey (4, 5) published interesting results along this line. Their first notes dealt with guinea pigs infected by virulent, semi-virulent or dead bacilli. In their latest paper (6), a small part of which treats of B.C.G., they have published graphs which show that, for doses of 0.001 or 0.0001 cc. of B.C.G., inoculated subcutaneously, intradermally or intraperitoneally, the skin sensitivity begins to manifest itself by a positive reaction to 0.01 cc. of tuberculin, about the eighteenth day after inoculation. It increases little by little until the end of the third or fourth month, at which time the testing dose has been lowered to 0.0001 cc. after which the sensitivity diminishes by steps.

We were interested in having more details about B.C.G. infected guinea pigs. Our animals are divided into groups injected with doses of B.C.G. stronger than those used by the above authors, the doses varying relatively little from one group to another, in order to study the correlations between small differences in the quantity of bacilli injected and the degree of allergy produced. It was thought worth while to show what happens among all the animals and this is why tables and graphs are presented to show the percentages of animals reacting to the different doses of tuberculin at successive periods after vaccination. The graphs relating to the most sensitive guinea pigs will be produced later. For the moment, we are not in position to say more than that our results are not in conflict with those of Boquet and Bretey.

The B.C.G. weekly emulsion, used for vaccinating new-born babies at the University of Montreal, is always prepared under the same rigorous conditions, and its composition may be regarded as constant. By means of guinea pig controls of these weekly emulsions of B.C.G. (*i.e.*, animals inoculated with the respective doses of 0.010, 0.005 and 0.0025 gm. of B.C.G.), three groups of about the same weight (400 gm.), the same age and of pale color, have gradually been formed. Before vaccination, these have been tested twice intradermally, with a one-week interval, with a dose of 0.1 cc. of a $\frac{1}{10}$ dilution of tuberculin from the Institut Pasteur. Tuberculin of the same lot and of constant activity was always used. The dilutions prepared the same day as the tests were made were calculated so that a 0.1-cc. dose might be administered. The syringe used was Dr. Kuss', made by Gentile Co. of Paris, and capable of delivering, by means of its cursor piston, $\frac{1}{50}$ cc. The animals' sides were epilated on surfaces 3 cm. in diameter.

After B.C.G. inoculation, the guinea pigs were tested about every five days at different points of their skin with a similar dose of tuberculin (*i.e.*, 0.01 cc.), until a positive response was obtained. These animals then received three doses of diluted tuberculin, *viz.* 0.001 cc., 0.0001 cc., and 0.00001 cc. at the same time but at different points of their skin. If any animals still reacted with the last dose, they were inoculated immediately with two smaller doses, namely 0.000005 and 0.0000025 cc. and so on, dividing the previous dose by two until the limit dose still active was obtained.

During the first two months, the tests were made about every week; during the third month, every two weeks; and from then on, once a month, by repeating the last positive dose and the next smaller one. For any dose of tuberculin, the positive reactions which turned negative were controlled by two subsequent tests at a week's interval.

The results were read at 24, 48 and 72 hours, and the positive reactions expressed as follows:

+ = well defined swelling of the skin of about 0.5 cm. in diameter.
++ = the same, but of 0.7 cm. in diameter.
+++ = the same, but of 1.0 cm. in diameter.
++++ = the same, but of 1.5 cm. in diameter.
+++++ = the same, but of 2 cm. and more in diameter.

When observed, the hemorrhagic or necrotic lesions were noted.

Similar experiments were carried out on a control group of at least ten non-vaccinated guinea pigs. The fact that we have never noted any positive or suspect reactions among this group assures us of the specificity of the reactions obtained among vaccinated animals.

Consecutive doses of tuberculin were never injected twice in the same area of the skin, when it was possible to avoid it, in order to eliminate the activation of homeotropic reactions, as described by von Pirquet (30) and

Weiss (31) for humans, although Boquet and Valtis (13) have demonstrated that, contrary to the opinion of Romer and Joseph (25, 26, 27), such an activation does not take place in guinea pigs as it may do in humans, since the guinea pig's skin is less sensitive to tuberculin than human skin.

To check this contention of Boquet and Valtis, their tests have been carried out on about 20 guinea pigs already in allergic state and on a few control animals. Varying doses of tuberculin were injected upon the seat of former reactions, which had given positive or negative results about seven days before, and at the same time on the same animals, similar doses were inoculated as a control in a new area of the skin. No activation or weakening of the reactions has been observed, either among the vaccinated or among the control group. Moreover it is generally admitted that tuberculin does not sensitize by itself.

Variations in weather do not seem to affect the reactivity of the skin. Even after brisk changes of temperature, atmospheric pressure and humidity, such as occurred during the past winter, our guinea pigs proved to be as sensitive as in hot weather. Nevertheless, we avoided making tests during very cold and moist weather.

Among twenty pregnant animals, a loss of skin reactivity a few weeks before and after delivery has been observed. The same phenomenon occurred during intercurrent diseases such as broncho-pneumonia, etc., that usually kill the guinea pigs. All these animals were discarded from the experiment and do not figure in the results. More than 2000 tuberculin tests have been made upon the 54 guinea pigs under experiment.

Table I shows how the weekly succession of vaccinations and the selection of subjects cause variations in the number of animals under experiment for every month after inoculation. Thus in July, at the beginning of our work, it was possible for us to test only two subjects in the group of guinea pigs subcutaneously vaccinated with 0.005 gm. of B.C.G. One of these was vaccinated a month before and the other two months before. In August, these animals, therefore, passed into the third and fourth months after vaccination. On the other hand, two newly vaccinated guinea pigs were examined in their first month of vaccination. The sum of the guinea pigs observed during the course of each monthly period following vaccination represents the total number on which our results for each corresponding period are based.

TABLE I

EXAMPLE OF THE PROGRESSIVE COMPOSITION OF THE MONTHLY GROUPS

—	Months after vaccination				
	1	2	3	4	5
July	0	1	1	0	0
August	2	0	1	1	0
September	3	2	0	1	1
October	2	3	2	0	1
November	4	2	3	2	0
December	0	4	2	3	2
Totals	11	12	9	7	4

It sometimes happens that a total monthly number is smaller than that of the preceding month since some animals, already negative to tuberculin, do not figure at this period. In this special case, the percentage of positive reactions was then calculated on the number of animals of the preceding month. In this way, these percentages are, if anything, lower.

Results

Allergy among Subcutaneously Vaccinated Guinea Pigs

Table II gives in round numbers the percentages of animals, vaccinated subcutaneously with the three doses of B.C.G., which have reacted to decreasing doses of tuberculin with an intensity of at least +. Data obtained so far cover a period of four months.

TABLE II

PERCENTAGES OF GUINEA PIGS, SUBCUTANEOUSLY VACCINATED WITH B.C.G., WHICH ACQUIRED HYPERSENSITIVITY TO DECREASING DOSES OF TUBERCULIN AT DIFFERENT PERIODS AFTER VACCINATION

Doses B.C.G., gm.		0.01 cc. tuberculin									0.0001 cc. tuberculin								
	Days	5	10	15	20	25	30	60	90	120	5	10	15	20	25	30	60	90	120
A 0.010	No. animals used	5	6	7	8	8	8	9	8	6	5	6	7	8	8	8	9	8	6
	No. giving positive response	1	2	5	7	8	8	9	7	5	0	0	0	1	2	3	6	4	3
	Reactors, %	20	33	71	87	100	100	100	87	83	0	0	0	13	25	37	66	50	50
B 0.005	No. animals used	7	7	10	10	11	11	12	9	7	7	7	10	10	11	11	12	9	7
	No. giving positive response	0	0	6	7	9	9	12	8	6	0	0	1	2	4	5	6	4	3
	Reactors, %	0	0	60	70	81	81	100	88	84	0	0	10	20	36	45	50	45	42
C 0.0025	No. animals used	4	4	4	6	6	6	8	5	5	4	4	4	6	6	6	8	5	5
	No. giving positive response	0	2	3	5	5	5	8	4	5	0	0	0	2	3	3	4	3	3
	Reactors, %	0	50	75	83	83	83	100	80	100	0	0	0	33	50	50	50	60	60
		0.001 cc. tuberculin									0.0001 cc. tuberculin								
A 0.010	No. animals used	5	6	7	8	8	8	9	8	6	5	6	7	8	8	8	9	8	6
	No. giving positive response	0	0	2	5	7	7	7	6	3	0	0	0	0	0	0	2	3	1
	Reactors, %	0	0	28	62	87	87	77	75	50	0	0	0	0	0	0	22	37	17
B 0.005	No. animals used	7	7	10	10	11	11	12	9	7	7	7	10	10	11	11	12	9	7
	No. giving positive response	0	0	4	5	9	9	8	7	6	0	0	0	0	0	0	0	0	1
	Reactors, %	0	0	40	50	81	81	75	77	84	0	0	0	0	0	0	0	0	14
C 0.0025	No. animals used	4	4	4	6	6	6	8	5	5	4	4	4	6	6	6	8	5	5
	No. giving positive response	0	0	2	3	5	5	7	4	4	0	0	0	0	1	2	4	3	2
	Reactors, %	0	0	50	50	83	83	87	80	80	0	0	0	0	17	33	50	60	40

Allergy Among Intraperitoneally Vaccinated Guinea Pigs

At the beginning of our experiments, a series of intraperitoneal inoculations in guinea pigs had already been completed and there were at hand a great number of these animals vaccinated with the three doses of B.C.G. mentioned above, the last vaccination having occurred four months previously. They had been tested with tuberculin previous to their vaccination and found negative.

It was thought to be of interest to experiment on them with the same method used for the subcutaneously vaccinated animals, despite the length of time since their vaccination, and the results of observation of this incomplete series are given here. The observation extended from the fourth to the tenth month after vaccination (Table III).

Another series of intraperitoneal vaccinations has recently been begun and data which will give an idea of the progression of hypersensitivity to decreasing doses of tuberculin during the first four months after intraperitoneal vaccination will soon be available.

Table III gives the numbers and percentages of animals vaccinated with the three doses of B.C.G. mentioned above, which reacted positively to decreasing doses of tuberculin with an intensity of at least +.

TABLE III

PERCENTAGES OF GUINEA PIGS, INTRAPERITONEALLY VACCINATED WITH B.C.G., WHICH HAVE ACQUIRED HYPERSENSITIVITY TO DECREASING DOSES OF TUBERCULIN IN PERIODS OF FOUR TO TEN MONTHS AFTER VACCINATION

Doses B.C.G., gm.		0.010 cc. tuberculin							0.0001 cc. tuberculin						
	Months	4	5	6	7	8	9	10	4	5	6	7	8	9	10
A 0.010	No. animals used	4	5	7	7	8	7	6	4	5	7	7	8	7	6
	No. giving positive response	4	5	7	7	8	6	5	4	5	7	5	6	6	5
	Reactors, %	100	100	100	100	100	86	83	100	100	100	71	75	86	83
B 0.005	No. animals used	4	6	7	6	6	6	6	4	6	7	6	6	6	6
	No. giving positive response	4	6	7	6	5	6	5	2	2	5	3	3	3	3
	Reactors, %	100	100	100	100	83	100	83	50	33	71	50	50	50	50
C 0.0025	No. animals used	5	6	9	10	9	9	7	5	6	9	10	9	9	7
	No. giving positive response	5	6	8	10	9	8	6	5	4	7	6	5	5	3
	Reactors, %	100	100	88	100	100	88	86	100	67	77	60	55	55	43
		0.001 cc. tuberculin							0.00001 cc. tuberculin						
A 0.010	No. animals used	4	5	7	7	8	7	6	4	5	7	7	8	7	6
	No. giving positive response	4	5	7	6	7	6	5	2	4	3	3	4	3	4
	Reactors, %	100	100	100	86	88	86	83	50	80	43	43	50	43	67
B 0.005	No. animals used	4	6	7	6	6	6	6	4	6	7	6	6	6	6
	No. giving positive response	4	5	6	5	3	5	4	0	2	4	1	1	2	0
	Reactors, %	100	83	86	83	50	83	67	0	33	57	17	17	33	0
C 0.0025	No. animals used	5	6	9	10	9	9	7	5	6	9	10	9	9	7
	No. giving positive response	5	6	8	8	6	8	4	1	2	3	2	4	4	3
	Reactors, %	100	100	88	80	67	88	57	20	33	33	20	44	44	42

Comments

A few facts observed may be pointed out with all the reserve necessary for unfinished work.

(*i*). Well-marked differences are noted from one guinea pig to another in the establishment and regression of the sensitivity to decreasing doses of tuberculin.

Theoretically the development of allergy in the most sensitive animal is characterized by the earliest reactions to smaller and smaller doses of tuberculin, which reactions are maintained for the longest period of time. In practice several guinea pigs which react quickly to small doses of tuberculin do not maintain these reactions as long as other guinea pigs of less quickly developed but more lasting sensitivity.

Fig. 6, which takes account as much as possible of the above theoretical definition, illustrates the individual curves of the most sensitive guinea pigs vaccinated subcutaneously. This is discussed under (*vii*). However, the following information shows that the actual classification of each individual as to its degree of allergy, may be effected at any time by *testing the animals with* decreasing doses of tuberculin.

(*ii*). The tables do not show the results obtained with doses of tuberculin smaller than 0.00001 cc. Among the guinea pigs subcutaneously vaccinated with the larger doses of B.C.G., the positive reactions to the minimum dose of 0.000005 cc. of tuberculin appeared only on two; on the 99th day for the animal which received 0.010 gm. of B.C.G. and on the 125th day for the one vaccinated with 0.005 gm. (Figs. 5 and 6).

On three animals vaccinated intraperitoneally with the quantities of B.C.G. mentioned above, very definite reactions for 0.000005, 0.0000025 and even 0.00000166 cc. of tuberculin have been sustained. These reactions were observed at about the sixth month and persisted at the tenth month.

(*iii*). In the light of the facts exposed, it is justifiable to assume that, when a positive reaction is obtained with small doses of tuberculin, a larger dose would also have produced a positive reaction, on the condition that the tuberculin be administered at the same time on the same animal and, as far as possible, at comparable points of the skin.

(*iv*). At any period after vaccination, the percentage of positive animals diminishes, in general, relative to the concentration of the dose of tuberculin. Thus at about the second month after vaccination, the percentage of positive animals vaccinated subcutaneously with 0.010 gm. of B.C.G., is about at its peak and is divided as follows:

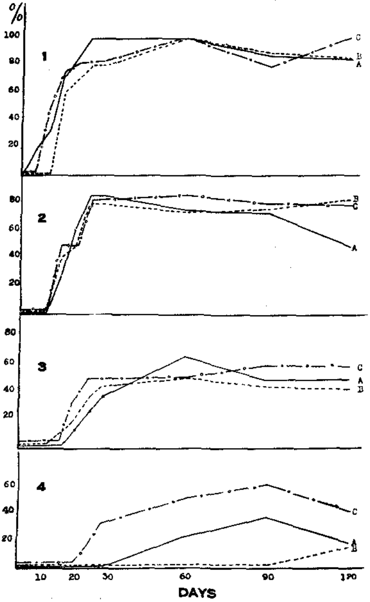
100% for 0.01 cc. of tuberculin.
77% for 0.001 cc. of tuberculin.
66% for 0.0001 cc. of tuberculin.
22% for 0.00001 cc. of tuberculin.

The animals may then be classified at any period according to their degree of allergy, by their response to decreasing doses of tuberculin, the animals reacting to 0.00001 cc. being considered the most sensitive.

(*v*). For a given dose of B.C.G. administered subcutaneously, the length of the ante-allergic period increases inversely as the concentration of the dose of tuberculin. The delay is greatest for the smallest doses.

(vi). The intensity of the reactions to tuberculin of the B.C.G.-inoculated guinea pigs varies certainly with the dose of tuberculin itself and with the length of time, but there is also a great variation in the intensity of the reaction which must be ascribed to the individual factor. The positivity of the reaction, however, is not affected by the individual factor to the same extent as the intensity.

For a given very sensitive animal, if the reaction to 0.01 cc. of tuberculin manifests itself with an intensity of 4+ or 5+, the reaction to 0.001 cc. will generally be 3+ or 4+; to 0.0001 cc. the decrease is more pronounced, 3+ or 2+ ; and finally to 0.00001, 2+ or simply +. However, there are several exceptions. The high doses of tuberculin often provoke reactions of equal intensity, but reactions more intense for a low concentration of tuberculin than for a high concentration have never been noted when the tests were made simultaneously at symmetric points of the skin.



FIGS. 1–4. *Data on guinea pigs subcutaneously vaccinated with doses of 0.010, 0.005, and 0.0025 gm. B.C.G. Variations in percentages of positive animals to an intradermal test of tuberculin for a period of four months' observation after vaccination.* FIG. 1. *Dose of 0.010 cc. tuberculin.* FIG. 2. *Dose of 0.001 cc.* FIG. 3. *Dose of 0.0001 cc.* FIG. 4. *Dose of 0.00001 cc. tuberculin.*

———— *Guinea pigs vaccinated with 0.010 gm. of B.C.G.*
— — — — *Guinea pigs vaccinated with 0.005 gm. of B.C.G.*
—o—o—o—o *Guinea pigs vaccinated with 0.0025 gm. of B.C.G.*

The ecchymotic or necrotic reactions have rarely been observed among the subcutaneously vaccinated animals. More frequently, however, among those vaccinated intraperitoneally these reactions were manifest for doses of 0.01, 0.001 and 0.0001 cc. of tuberculin and occurred irregularly even at ten months after vaccination.

On account of the great individual variation and the periodic irregularities which make it useless for comparative appreciation, the intensity of the tuberculin reaction seems to us, for the present time, but an accessory pointer in the measure of the hypersensitivity to decreasing doses of tuberculin in guinea pigs vaccinated with the given doses of B.C.G.

(*vii*). The study of the accompanying tables and percentage graphs drawn up for subcutaneously vaccinated guinea pigs, reveals certain interesting facts concerning the establishment and development of allergy among all the animals, for decreasing doses of tuberculin.

Five days after vaccination, there was an animal positive to 0.01 cc. of tuberculin among group A, vaccinated with the highest dose of B.C.G. During the course of the first ten days after vaccination, while the percentage of animals positive to this dose of tuberculin increased substantially for groups A and C, *no guinea pig in group B had yet reacted.* But at the end of 15 days, nearly three-quarters of the animals in each group reacted positively without great distinction of percentages among the three groups. At this period, the same animals reacted to 0.001 cc. of tuberculin for an average of about 40%; but just one animal in the three groups has reacted to 0.0001 cc. and none to 0.00001 cc., to date. There is no apparent correlation between the variations in percentages and the differences in the quantities of B.C.G. received.

At the end of the twentieth day, a good step is made in the establishment of allergy among *many* guinea pigs, as may be judged by the increase in percentages. It happens that the animals responding earliest to the dose of 0.00001 cc. of tuberculin, that is about the 25th day, are in group C, vaccinated with the smallest dose of B.C.G. However, two guinea pigs in groups A and B, as already mentioned, have given positive reactions for a dose of 0.000005 cc. of tuberculin at about the fourth month after vaccination.

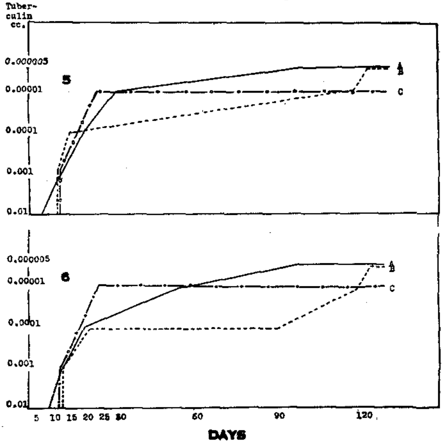


FIG. 5. *Earliest positive reactions to decreasing doses of tuberculin in each group of guinea pigs, subcutaneously vaccinated with 0.010, 0.005, and 0.0025 gm. of B.C.G.* FIG. 6. *Individual logarithmic curves showing the development of hypersensitivity to decreasing doses of tuberculin of the most sensitive guinea pig in each group of vaccinated animals.*

——— *Guinea pigs vaccinated with 0.010 gm. of B.C.G.*
— — — *Guinea pigs vaccinated with 0.005 gm. of B.C.G.*
—o—o—o—o *Guinea pigs vaccinated with 0.0025 gm. of B.C.G.*

Fig. 5 expresses clearly the relation between the three groups of animals vaccinated subcutaneously, as it pertains to the precocity of the positive *reactions to decreasing doses of tuberculin.* Thus among these animals, the

test for 0.01 cc. of tuberculin may become positive about the fifth day after vaccination; the test for 0.001 cc., at about the twelfth day; 0.0001 cc. the 15th day; the positive reactions for 0.00001 cc. may appear at the end of the first month or during the second and third months; and finally, the test for 0.000005 cc. does not give positive results before the fourth month following vaccination.

Fig. 6 represents the curve of the most sensitive animal in each group, and illustrates how the sensitivity to decreasing doses of tuberculin may vary from one animal to another. It also demonstrates the apparent independence of the sensitivity to small variations in the dose of B.C.G. administered subcutaneously.

If one relies on the percentages, one sees that the maximum percentage of positive reactions among subcutaneously vaccinated guinea pigs is reached *at about the second month after vaccination for the higher doses of tuberculin*, *i.e.*, 0.01 cc., 0.001 cc., and 0.0001 cc. For the dose of 0.01 cc., the percentage reaches 100 in each group.

After this period, a certain number of guinea pigs, about 15% in each group, cease to respond to 0.01 cc. of tuberculin, and the curves of Fig. 1 *come down gradually without showing any distinction between the doses of* B.C.G. received. In article (*ix*) may be found the probable explanation of the sudden rise of curve C during the fourth month. During this month the sensitivity to 0.001 cc. and 0.0001 cc. of tuberculin still persists among the majority of the animals, the percentages being the same as in the second month.

The maximum percentage of reactions positive to 0.00001 cc. of tuberculin, namely an average of 48%, is seen to be reached only during the third month for groups A and C; in group B, only one guinea pig gives such a reaction and it happens during the fourth month. As already mentioned, the same fact is observed concerning the reactions to 0.000005 cc. of tuberculin which were produced in two guinea pigs not before the fourth month. We would like to call attention here to the marked delay in the appearance of the sensitivity to the minimum doses of tuberculin in many of our guinea pigs.

As already stated in article (*iv*) the tests with doses of tuberculin smaller than 0.01 cc. permit a classification of our guinea pigs as to their state of hypersensitivity.

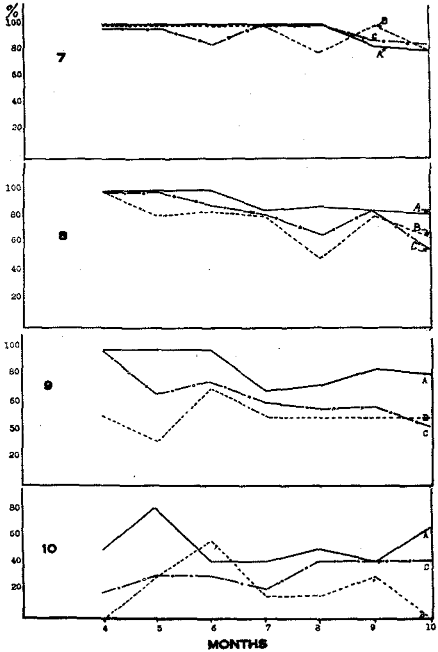
(*viii*). Concerning the animals vaccinated intraperitoneally, no other comments than the following seem justified. *At the fourth month after vaccination*, that is when the tests began, all the animals reacted to 0.01 cc. of tuberculin; the majority still persist in this state at the tenth month. As concerns the other doses of tuberculin, the decrease in percentages has already begun during the fifth and sixth months and continues slowly with intermittent returns.

Table III shows that, while 100% of animals react positively to 0.01 cc., 0.001 cc. and 0.0001 cc. of tuberculin, the percentage of positive reactions

to 0.00001 cc. is much lower. This last test has probably selected the most sensitive guinea pigs among which the doses of 0.000005, 0.0000025 and 0.00000166 cc. may now permit another selection. (See article (*ii*)).

Here, as among the subcutaneously vaccinated animals, the sensitivity to 0.00001 cc. of tuberculin seems retarded for many animals, since the average maximum percentage of positivity to this dose is reached during the fifth and sixth months, whereas for the remaining doses of tuberculin, the maximum was already reached during the fourth month. The group of 0.010 gm. stands out clearly from the other two, by its high percentage of positive reactions, especially for doses of tuberculin less than 0.01cc.

ix. The regression of sensitivity seems to carry an individual factor still more than its establishment, since the percentage curves decline less rapidly than they rise. In some animals, however, the sensitivity to a given dose of tuberculin is seen to disappear for a certain period, to reappear later on. These irregular recurrences explain the sudden rises of certain curves during the period of decline. This should warn us against too rapid and final a classification of the negative animals.



FIGS. 7–10. *Data on guinea pigs vaccinated intraperitoneally with doses of 0.010, 0.005 and 0.0025 gm. of B.C.G. Variations in percentages of positive animals to an intradermal test for periods of from 4 to 10 months' observation after vaccination.* FIG. 7. *Intradermal test, 0.01 cc. tuberculin.* FIG. 8. *Tested with 0.001 cc. tuberculin.* FIG. 9. *Tested with 0.0001 cc. tuberculin.* FIG. 10. *Tested with 0.00001 cc. tuberculin.*

———— *Guinea pigs vaccinated with 0.010 gm. of B.C.G.*
– – – – – *Guinea pigs vaccinated with 0.005 gm. of B.C.G.*
—o—o—o—o *Guinea pigs vaccinated with 0.0025 gm. of B.C.G.*

Sufficient data to make any more comment on the regression of sensitivity are not yet available. Measurement of the length of its persistence for all doses of tuberculin is now planned.

Summary

1. A quantitative study of the allergic state of guinea pigs vaccinated with 0.010, 0.005 and 0.0025 gm. of B.C.G. has been sketched as a preliminary to researches on their state of immunity.

2. By serial and intradermal reactions with decreasing doses of tuberculin, the measurement of the degree of allergy of these animals at different periods after inoculation has been undertaken, as has also the determination of the development of this hypersensitivity.

Acknowledgment

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THE LIFE CYCLE OF *FASCIOLOIDES MAGNA* (BASSI, 1875), THE LARGE LIVER FLUKE OF RUMINANTS, IN CANADA

WITH OBSERVATIONS ON THE BIONOMICS OF THE LARVAL STAGES AND THE INTERMEDIATE HOSTS, PATHOLOGY OF *FASCIOLOIDIASIS MAGNA*, AND CONTROL MEASURES[1]

By W. E. Swales[2]

Abstract

The life history of *Fascioloides magna* in Canada has been elucidated, the intermediate hosts being two fresh-water gastropods, *Fossaria parva* (Lea) and *Stagnicola palustris nuttalliana* (Lea). The morphology and bionomics of the egg and larval stages are described, particular attention being given to the non-parasitic stages. The ecology of the gastropod hosts in Canada is briefly described.

A histopathological study of the lesion in definitive hosts reveals that this parasite in large Bovidae causes a severe tissue reaction. The lesion in these animals is generally in the form of a closed fibrous cyst from which eggs are unable to pass, and thus the life cycle cannot be completed. In Cervidae, the cavity in the liver is connected directly with the bile duct system, and there is a free egress of ova. From these facts it is inferred that this parasitic disease can only occur in the presence of Cervidae.

Laboratory animals have been artificially infested with maritae, thus extending the host records of the trematode.

A brief historical review, a summary of the present knowledge of distribution and definitive hosts affected, and a description of the control measures, are included.

Introduction

Fascioloides magna, the digenetic trematode which forms the basis of this study, is essentially of North American origin but has been introduced into Europe in imported animals. It is an extremely important helminth parasite of ruminants both from a pathological and economic viewpoint. It is unlikely that it will ever be as widespread in its distribution as *Fasciola hepatica*, with which it has so often been compared, but it has been described as being of equal importance in some parts of North America.

In spite of being native to North America this trematode was first described by Bassi from various deer enclosed in a national park in Turin, Italy. It is generally agreed that the Italian epizootic was the result of importations of American wapiti. The parasite had, however, encountered a satisfactory intermediate host in Europe because several ruminants of other species were infested at that time and it has since been reported from southern Germany. Owing to its peculiar location in the liver tissue of its host and the outward similarity of the lesion to a common abscess, it is probable that it has been overlooked in many cases.

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This parasite was the subject of an extensive morphological study by Stiles in 1894. In this work the author included an excellent historical review of the species and elucidated its synonymy. Stiles also included notes on the pathological effect upon the definitive host and gave a comparative description of the egg and miracidium. He stated that the life cycle was probably very similar to that of *F. hepatica* and experiments were being conducted at that time in order to determine the different stages in an intermediate host. It was not until very recently, however, that an intermediate host was reported by Sinitsin (1930) but now three more experimental snail hosts have been recorded by Krull (1933), the latter during the later stages of this work.

Hadwen (1916) and Cameron (1923) recorded this fluke from ruminants in British Columbia and Alberta respectively. The records of the National Parks Branch of the Department of the Interior of Canada revealed in 1931-32 that infested ruminants in the Wainwright area of Alberta were becoming more numerous. With the co-operation of the National Parks Branch and the Health of Animals Branch, the writer undertook a survey of the endemic area and made preliminary investigations into the life cycle. It was then noted that the snail fauna was peculiar in that very few members of the group recorded as hosts for other fasciolinid trematodes were present. No naturally infested snails were obtained although it seemed probable that one snail (*Stagnicola palustris nuttalliana* Lea) was a natural host.

Owing to the advanced season and the limited time available, further investigations were confined to records of the prevalence of the parasite, the determination of ecological observations upon the definitive hosts and the practicability of future control measures on the observed area.

Following the establishment of the Institute of Parasitology at Macdonald College in 1932, it was decided to continue the investigations. In 1933 and 1934, with the whole-hearted support and co-operation of the National Parks Branch, the life cycle of this trematode on the endemic area was elucidated and a fairly complete study of the bionomics of the parasite was completed. Thereafter, control measures were applied to the area most affected, and it is confidently expected that this parasite will be kept under control in the regions of heavy infestation.

Historical Review and Notes on Distribution and Definitive Hosts

To present a full historical review of *Fascioloides magna* is merely to repeat Stiles' excellent account in his paper on the anatomy of this parasite. However, for the benefit of those to whom Stiles' paper is not now available, a brief historical summary taken chiefly from Stiles, but with a few additional notes, is given here.

Bassi, in 1875, observed an epizootic among deer of the Royal Park near Turin, Italy, the symptoms being typical of the well known fascioliasis in sheep. He gave an unsatisfactory description of the parasite present, naming

it *Distomum magnum*. He believed that it was introduced into the park in American Wapiti (*Cervus canadensis*). Most workers did not accept Bassi's species because of his poor description, but it was accepted in 1882 by Perroncito who described it as "*Distoma grande*, (*Distoma magno*, *Distoma magnum* Bassi)."

In 1887, Curtice recorded this trematode in cattle in Kansas, U.S.A., and while at first identifying it as *F. hepatica*, later said it was *F. magna*.

From 1889 to 1892, this parasite was recorded from the United States by Dinwiddie, Osborne (1890), Curtice, Francis (1891) and Leidy. Hassall, 1891, described it as a new species, under the name *Fasciola carnosa*, but finding the specific name already applied to another distome, changed it during the same year to *Fasciola americana*. In the same year, Francis described it as *Distomum texanicum*, being advised by Leidy who also had overlooked Bassi's paper. Leidy himself later considered the form as identical with *Distoma crassum* which he had found in the intestine of a Chinese boy; this was later shown to be quite a different species.

Stiles, in 1892, pointed out that Francis' species was identical with *F. americana* Hassall, and was probably the parasite originally described by Bassi; he later compared specimens and proved this hypothesis. Just before this Leuckart compared an American specimen with one of Bassi's original specimens and declared them identical. Stiles (1891) had shown that the specific name *magna* should be retained for Bassi's species, and thus the Italian specimens reported by Bassi, Perroncito and Sonsino (1890) and the American species described by Hassall and Francis were henceforth recognized under the name *Fasciola magna* (Bassi, 1875).

In 1917 Ward showed that, owing to the lack of the distinct anterior cone and the fact that the vitellaria are confined to the region ventral to the intestinal branches, the suggestion of Odhner that this form should constitute a new genus should be followed. He therefore named a new genus *Fascioloides*, designating as the type *Fascioloides magna* (Bassi, 1875) Ward, 1917.

The synonymy is now:—

Distomum magnum Bassi, 1875.
Distomum hepaticum Curtice, 1882.
Fasciola hepatica Dinwiddie, 1889. (Nec. Linn. 1758).
Fasciola carnosa Hassall, 1891.
Fasciola americana Hassall, 1891.
Distomum texanicum Francis, 1891.
Cladocoelium giganteum Stossich, 1892.
Fasciola magna (Bassi, 1875) Stiles 1894.

As stated elsewhere in this paper, the fact that the external appearance of the liver lesion caused by *F. magna* is totally unlike that caused by other liver flukes, may account for the scanty records in some parts of North America. Owing to the danger of contaminating equipment, large abscesses in the liver of cattle are not incised during meat inspection and this fact would account

for the scanty number of records in Canadian cattle. The fact that this fluke is of such prevalence and importance in cattle in the southwestern United States, indicates that it is at least a great potential danger to Canadian cattle. It is common in many wild and semi-wild ruminants in Alberta and British Columbia, and this fact indicated that it only needed the introduction of the ova into many cattle-raising districts to produce conditions similar to those described by Stiles in the United States.

In British Columbia, reports issued from time to time indicate that deer on the coast are badly affected by liver fluke disease. Mr. F. R. Butler, Inspector of the Game Commission of that province, informs me that there is from time to time mortality among deer, principally on Vancouver Island, as a result of liver fluke. Many deer from these regions have been examined by authorities, and specimens of liver flukes, all of which are *F. magna*, have been forwarded to me. This was the locality of the first recorded case in Canada, and it is apparent that the reports from this district at least indicate the prevalence of the trematode in the province.

Records of distribution in the United States, including those summarized by Hall in 1912, show that *F. magna* occurs in the states of Texas, Kansas, Arkansas, California, Illinois, Iowa, Minnesota, Michigan, Montana, Oklahoma, Wisconsin, Colorado (?) and New York. In Canada, records already published indicate that the fluke is confined in distribution to British Columbia and Alberta. However specimens from *Odocoileus virginianus* in eastern Ontario have recently been received. This record considerably extends the distribution in Canada, but it is to be expected that the parasite will be recorded from other provinces.

The recorded definitive hosts are as follows:—

UNITED STATES OF AMERICA			
Cattle	(*Bos taurus*).	Sheep	(*Ovis aries*).
Wapiti	(*Cervus canadensis*).	(?) Goat	(*Capra hircus*).
Moose	(*Alces alces americana*).	Horse	(*Equus caballus*).
Deer	(*Odocoileus virginianus*).		
ITALY			
Sheep	(*Ovis aries*).	Fallow deer	(*Dama dama*).
Wapiti	(*Cervus canadensis*).	Stag or Red deer	(*Cervus elaphus*).
Blue bull	(*Boselaphus tragocamelus*).	Sambur	(*Cervus unicolor*).
GERMANY			
Red deer	(*Cervus elaphus*).		
CANADA			
Bison	(*Bison bison*).		
Coast deer	(*Odocoileus columbianus*).		

to which I am able to add:—

Yak	(*Bos grunniens*).	Virginia deer	(*Odocoileus virginianus*).
Hybrid, Domestic×Bison	(*Bos taurus*×*Bison bison*).	Mule deer	(*Odocoileus hemionus*).
Cattle	(*Bos taurus*).	Wapiti	(*Cervus canadensis*).

In addition to the above-mentioned naturally infested hosts are the following which were artificially infested during the course of this study.

Sheep	(*Ovis aries*)
Domestic rabbit	(*Lepus cuniculus* var. *domesticus*)
Guinea pig	(*Cavia porcellus*).

Neveu-Lemaire refers to *F. magna* as a parasite of cattle, sheep and goats, but the reference to the last host is obscure. At least one obscure record of this parasite in goats in the United States is present in old literature, but an uncertainty exists as to the origin and identification of the specimens. There is little doubt, however, that *Capra hircus* could be infested.

Snail Hosts with Notes on Their Ecology

In order to determine the intermediate host of *F. magna* in Canada an endemic area in Alberta was selected and a thorough survey of all the aquatic and semi-aquatic gastropods was undertaken. This area is situated in the vicinity of Wainwright and comprises approximately six thousand acres; it is part of a National Park and its fences enclose herds of *Bison bison*, *Bos grunniens* and *Cervus canadensis*. The young animals of the last-mentioned herd were heavily infested with the trematodes and have had access to no other grazing lands; many showed clinical symptoms of fascioloidiasis.

The area is typical "Western parkland", rolling prairie with sandy soil and scanty tree life composed of scrub poplar bluffs. A lake, about one square mile in area, is situated near the centre of the main enclosure; this is fairly "alkali", has well-defined sandy shores and harbors little or no vegetation and hence no snails could be found in its environs. One other permanent lake is present which is not one of the so-called alkali lakes. It covers approximately one hundred acres, is surrounded by a shore-line rich in vegetation and contains a snail fauna of four species, *Stagnicola palustris nuttalliana*, *Lymnaea stagnalis wasatchensis* and two *Gyraulus* spp. The water in mid-summer is only slightly alkaline (pH 7.5–8.0).

The other watered areas are 32 small swamps, muskegs and sloughs, only six of which are permanent, and a small sandy-shored lake which contains no snail life. The water in the above-mentioned areas varies in alkalinity from pH 8.5 to pH 9.5 and is stagnant. The permanent, and some of the semi-permanent, swamps and sloughs were rich in snail life composed of the following species, listed in approximate order of prevalence.

1. *Stagnicola palustris nuttalliana* (Lea).
2. *Aplexa hypnorum tryoni* Currier.
3. *Lymnaea stagnalis wasatchensis* Hemphill.
4. *Physa gyrina* Say.
5. *Succinea retusa* Lea. (On surrounding soil.)
6. *Gyraulus circumstriatus* (*Tryon*).
7. *G. umbilicatellus* (*Cockerell*).
8. *G. deflectus obliquus* (DeKay).
9. *G. crista* (Linn.)
10. *Helisoma trivolvis* (Say).
11. *H. subcrenatum* (Carpenter).
12. *Menetus exacuous megas* (Dall).

S. palustris nuttalliana is by far the most prevalent of the snails present and is the only species found in three sloughs which were observed for three consecutive years and which were completely dry in mid-July of each summer. Often more than fifty specimens of this snail have been collected with one three-foot sweep through the vegetation with a five-inch scoop.

In addition to these, there are four small areas where there is a spring-time accumulation of water which is never of great volume but is contained in an assembly of small depressions amid seepy land and is covered by sedges (*Carex* spp.). These areas altogether do not comprise more than two acres, three of them are natural drainage ways leading to a permanent slough or lake in each case. They are completely dry in the second or third week of June in normal years and, during preliminary investigations, were not considered as possible snail habitats owing to the fact that this work was undertaken in July. Later investigations showed that, in May, these areas harbored *Fossaria parva* (Lea) in small numbers. These snails were found in animal tracks effectively covered by sedges and were difficult to find. They were feeding on dead sedge leaves and at times were found to be actively crawling over the mud sides of the tracks. The largest population observed was fifty snails on an area of approximately 200 sq. ft.

Cercariae of *F. magna* emerged from one naturally infested specimen of *F. parva* on the twentieth day after collection, and from four other specimens of this species on the twenty-fourth day. From all of these snails experimental definitive hosts were successfully infested with maritae. Following these findings, particular attention was paid to the habitats of *F. parva*. Towards the end of July an attempt was made to determine the level to which the snails had burrowed when their habitat dried up. It was found that young snails could be recovered from the first two six-inch layers of the soil over their spring habitat. The majority appeared to be at a depth of six inches, under soil which was well covered with dried sedges. These observations were made by a co-operator, on behalf of the writer, and were not sufficiently extensive to indicate definitely the means employed by these animals to withstand adverse conditions. It is probable then, that the cycle of the trematode within the snail must be completed under natural conditions in the short period between the thawing and drying of the upper soil of the areas under study in Alberta. Alternatively, the intramolluscan stages of the parasite may live over the dry summer months and the winter in the tissues of the snail, their activity and development corresponding to that of their host.

In addition to the *F. parva*, specimens of all the other snails were returned to the Institute and kept under observation in aquaria. Cercariae emerged from one naturally infested specimen of *Stagnicola palustris nuttalliana* on the sixty-ninth day following collection. Nine of the encysted metacercariae were fed to a rabbit which was successfully infested with two maritae of *F. magna*. This snail was one of 400 kept under observation, the remainder being negative on subsequent examination.

No other species of snail was found to be naturally infested; artificial infestation was also unsuccessful in spite of the fact that miracidia readily attacked *Aplexa hypnorum tryoni*, *Physa gyrina* and *Lymnaea stagnalis wasatchensis*.

Of 50 specimens of *F. parva* kept under observation, five were found to be naturally infested. From one of these specimens 687 cercariae emerged and encysted during a period of 58 days.

Two species of snails were thus shown to act as natural intermediate hosts for *F. magna* in Canada. *F. parva* (Lea) was found to be living in small areas which are wet and swampy in the spring time but are completely dry during summer and autumn. It apparently burrows into the soil in order to survive the unfavorable dry seasons. It feeds on the limited amount of decaying vegetation, composed chiefly of sedge leaves and grass. This species is very active, and is often found on the mud surrounding water-filled animal tracks which contain most of the free water in May.

S. palustris nuttalliana (Lea) is very different from *F. parva*, both from the ecological and morphological points of view. It is at present recognized as a sub-species of *S. palustris* (or *Lymnaea palustris*, under which name it is still recognized in some parts of the world), but may be a geographical strain. It inhabits stagnant bodies of water which contain large quantities of vegetation, both living and dead. These waters may be permanent or semi-permanent, and on the endemic area are always fairly warm and have a large range of alkalinity. Sloughs that dry up in the spring are not favorable habitats, but those that contain water until midsummer are very suitable. It is often found in company with *L. stagnalis wasatchensis* Hemphill, in small lakes containing abundant vegetation. Unlike *F. parva* it is not found out of the water, and prefers to remain under the surface of its stagnant water habitat. Although only one specimen of this snail has been found to be infested under natural conditions, it can easily be infested artificially, and is able to act as an intermediate host throughout the cycle. It is probable that examination of a great number of specimens from an infested area would reveal a number of naturally infested individuals.

The inclusion of *S. palustris nuttalliana* in the group of Lymnaeidae known as intermediate hosts of fasciolinid trematodes in North America is important. It means that snails of this universally prevalent species must be considered as possible vectors of *F. magna*, even where snails of the other groups have been destroyed. Control measures must always be modified accordingly, but more strict attention must be given to the warning of Baker, that promiscuous applications of copper sulphate may have untoward effects. This snail also lives normally in lakes inhabited by fish of several species and in waters suitable for fish spawning grounds.

In addition to the snails shown in this work to be intermediate hosts in Canada, are *Galba bulimoides techella* (Halderman), *Fossaria modicella* (Say), *F. modicella rustica* (Lea) and *Pseudosuccinea columella* (Say). The first one, which has not yet been recorded in Canada, was reported by Sinitsin as a host in Texas, U.S.A., and the other three recently reported as artificially infested species by Krull (1933) in the United States.

G. bulimoides techella, *F. modicella* and *F. modicella rustica* are commonly found in intermittent pools or streams and have not been recorded in habitats similar to those here described for *F. parva*. They may be found in company with *S. palustris nuttalliana* but the more common habitat of this snail would

not be suitable for their common existence. These three species were not present on the small endemic area under study, but *F. modicella* was found in small numbers in a spring-fed pool about seven miles away.

P. columella is known as a "quiet bay or pond type" and is found in company with *L. stagnalis*. It was not present in the Alberta area but it may be found in the same habitat as *S. palustris* (or *Stagnicola* spp.) in other areas.

The Intermediate Hosts in Aquaria

F. parva was kept in shallow nine-inch specimen jars in two to three inches of tap water. Dried sedge leaves, moss and filamentous algae were added, and dried lettuce leaves were supplied as food. The water was completely changed twice per month but fecal matter was siphoned off twice a week. Sand and mud were supplied to two jars but this apparently made no difference to the health of the animals. Air was continually bubbled through the water in each jar by means of a series of glass tubes connected to a German K D A air pump.

A second generation of this species was observed after forty days in the aquaria and these specimens were used for artificial infestation experiments, when they were over four days old. Even when snails of these species were extremely small they were able to withstand infestations and to produce cercariae normally. No death from the effects of the parasites has ever been observed, which leads to the belief that this species is the normal intermediate host in Canada.

S. palustris nuttalliana was kept in large jars which were aerated as already mentioned, but were only cleaned once in two months, tap water occasionally being added to replace that lost by evaporation. These snails quickly removed growing algae if in large numbers, but did not appear to suffer from its consequent absence. They were fed on dried lettuce leaves and dried sedges, and consumed large quantities of the former when it was supplied in a dried condition which speeded its decomposition in the water.

A second generation of these snails did not appear until the adults had been in the aquaria for 65 days. In the first attempts to infest this species many of the snails died on the third and fourth days, but this phenomenon did not re-occur in further tests, so that it is probable that some other factor lowered the resistance of the snails.

A factor that was at first considered important was the hydrogen ion concentration of the water in aquaria. Tests of water in aquaria showed that *F. parva* lived normally in water ranging from pH 6.5 to pH 8.5 and that *S. palustris nuttalliana* lived in waters ranging from pH 5.5 to pH 9.5. These figures agree on the alkalinity side with the findings in their natural habitat. The pH of the water in the aquaria was subsequently never corrected, and there were no ill effects upon the animals.

It was found desirable to add a small lump of calcium sulphate to each tank in order to supply the calcium requirements of the snails. This apparently increased the growth rate of the young snails.

The shell of laboratory-raised specimens of *F. parva* is somewhat darker in color than that of the snails in their natural habitat. On the other hand, the shell of *S. palustris nuttalliana* becomes quite light in color after about one month in the tanks.

The temperature of the general aquarium room could not be accurately controlled, but the mean temperature throughout the experiments was 24°C.

Artificial Infestations

For the purpose of determining the time required for completion of the intramolluscan stages in *F. parva*, five embryonated eggs per snail were suspended in a small vial in the jar containing the snails under experiment. In this way, the hatching time and hence the approximate time of infection was determined by frequent examination of the ova in the vial. In the first two tests the twelve snails in each were all infested and the cercariae commenced to emerge on the forty-ninth and fifty-fifth day, respectively. On the day of the first appearance of metacercariae on the walls of the test jars, each snail was placed in a separate jar and the date of cercarial emergence was noted. The periods ranged from 49 to 58 days from the day of miracidial attack.

Second generation *S. palustris nuttalliana* were exposed in a similar way, but in the first three lots of 12 snails the development of the sporocyst did not appear to continue after the fourth day. In one other lot of ten snails, eight specimens subsequently produced cercariae and the first encysted metacercariae were noted on the walls of the tank on the fifty-seventh day.

Pre-parasitic Stages in the Life Cycle

The Egg

The eggs of *F. magna* are passed out of the normal fluke cavity in the host's liver tissue by way of the small bile ducts, which are merely intercepted in their course and are not closed off by the formation of the normal fluke cavity. From here they progress normally, passing into the alimentary canal by way of the main bile duct. In long standing cases, or in heavy infestations, some of the fibrous cavities are completely cut off from the bile system and are then non-functional. No infested Bovidae have been found to be evacuating eggs in the feces. One animal (*Bos grunniens*) was found to be infested with 12 adult flukes in completely closed fibrous cavities and had 24 other cavities in which only dead and decomposed flukes were observed. Of all the eggs which were present in a heavy suspension in the melanoid fluid, less than 0.1% developed in culture. In one *Cervus canadensis*, which harbored 15 live flukes in nine "open" cavities and two "closed" cavities, eggs were passing out of the main bile duct in huge numbers, the formolized duct yielding 497 eggs per millimetre of length.

One other test animal (*Cervus canadensis*) which harbored 20 live adult flukes, all in normal open cavities, was found by four tests to be passing an average of 37.41 eggs per gm. of feces. Only one adult animal of this species could be confined for the purpose of obtaining the number of eggs per day per fluke but rough estimations indicate that this figure would be between six and seven thousand.

These findings do not agree with those of Sinitsin, who on two occasions has stated that the eggs probably pass in some unexplained way into the blood stream, because he has found no communication between fluke cavities and bile ducts. It is apparent that Sinitsin was working with animals that had reacted to the infestation to such an extent that the fluke cavities were closed by a complete fibrous cyst. This view is supported by the fact that he was working with *Bos taurus*, obviously an abnormal definitive host for *F. magna*, which, it is generally agreed, is of North American origin. The writer has noticed this condition on two occasions in *Bos grunniens*, but has always found a heavy egress of eggs in *Cervus canadensis* and *Odocoileus* spp.

Morphology of the Undeveloped Egg

The size and shape of the ova vary considerably, the most usual specimen being 148μ long by 94μ wide and ovoid in shape. In order to indicate the variations in size more clearly than is possible by giving extreme and average measurements, the length and width curves from the measurements of 200 ova were plotted (Fig. 1). These were taken from a mixed lot of fresh feces of *Cervus canadensis*, and the figures obtained agree closely with all the writer's previous findings. It will be noted that the variation in length is much greater than in width, and that greater abnormalities are liable to occur in the length. The normal egg size as indicated by the chart is not the average size.

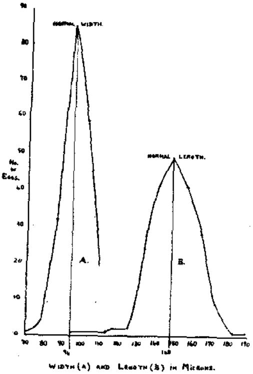

FIG. 1. *Chart showing the variation in size of the ova of F. magna.*

Eggs containing fully formed miracidia vary very considerably in shape; probably the activity of the miracidium causes lateral distention or elongation in many cases. It has been noted in many cultures, kept in deep stagnant water to prevent hatching, that the eggs tend to become broader. Many of these appear similar to the abnormal shapes of the eggs of *Fasciolopsis buski* as pictured by Barlow (1925).

The size of mature eggs teased from the uteri of live flukes does not appear to vary in relation to the size of the trematode. The developmental period of these eggs is the same as that of eggs freshly passed from the host's alimentary canal.

The peculiar appendage on the anti-opercular end of the egg as described by Sinitsin (1933) is present in varying forms on practically all eggs removed from the fluke cysts in the liver. It is only present on approximately 20% of eggs passed normally from the host. This appendage protrudes from a small pore which is situated slightly to one side of the middle line of the egg; it varies from 4 to 21μ in length. The findings of the writer indicate that it is an irregularly shaped mass of protoplasm which has been extruded through the pore, probably by some pressure within the egg while it is in the uterus of the trematode. The appendage is often lost if the eggs are forcibly screened through fine sieves, which may explain why this feature was not noted by Stiles (1894) in his description of the egg.

The writer cannot agree that the eggs of *F. magna* are lighter in color than those of a *Fasciola*. The eggs of *F. hepatica* are aptly described as being of a delicate light brown color; those of *F. magna*, normally evacuated from the host, are golden brown and somewhat more opaque. They are easily distinguished by this darker coloration from normally evacuated eggs of *F. hepatica* of sheep, as well as by their larger size and by the presence, in some of them, of the afore-mentioned appendage. In addition to these distinguishing features there is a marked thickening of the shell at the anti-opercular end, varying from one and a half to three times the thickness of the other parts of the shell. The operculum is irregularly rounded and from 15 to 20μ in diameter.

Freshly evacuated eggs have an undivided germ cell and approximately forty yolk globules which are somewhat more opaque but otherwise very similar to those of *F. hepatica* and to the descriptions of *F. buski*. The germ cell is difficult to observe, being smaller than that of *F. hepatica*. After the eight-cell stage, the development is even more difficult to follow until the morula appears; however, no observations have been made that show any difference in comparison with the other closely related trematodes.

The miracidium develops quickly, cilial motion being observed some time before the eye spot appears. The mucoid plug grows rapidly until the miracidium has reached its full development, by which time yolk globules have been broken up and the oily substance has collected into two masses.

The miracidium presents a cramped appearance in the egg, being larger than in related species. The anterior end is abruptly curved over between the first and second quarter of the body's length. In the animal's struggles at hatching time the anterior end is straightened and the mouth is directed into the mass of the mucoid plug, which in this case is readily flexible. The body then takes a rough "V" shape, and the oily masses are displaced.

Development of the Egg

The eggs commence to develop as soon as they are passed into suitable conditions of temperature and moisture. In the summer time in western Canada they are ready to hatch in 35 days, even though they remain in damp feces. Many of the eggs taken from "closed" cysts in the liver do not develop at all, or if they do, they take much longer than normally evacuated ones.

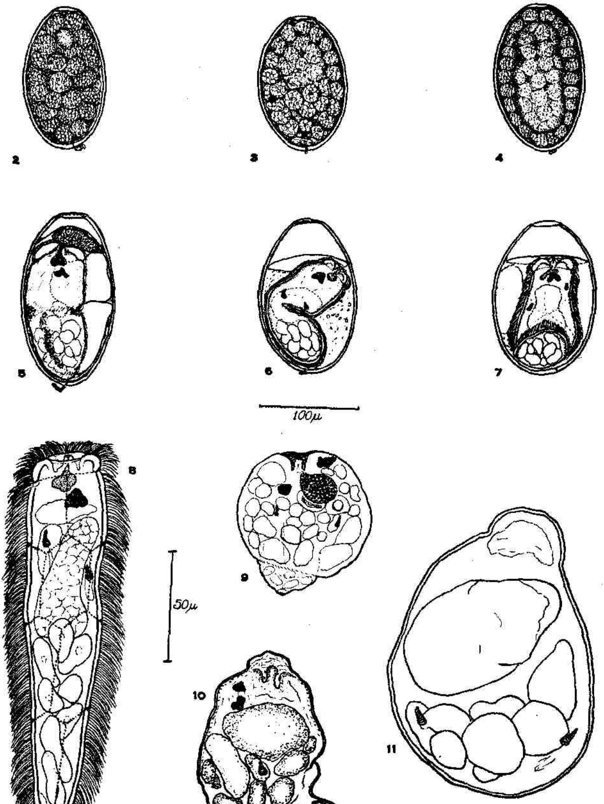

FIG. 2. *An ovum in the one-cell stage showing the germinal area and the arrangement of the yolk balls.* FIG. 3. *An ovum in the 16-cell stage.* FIG. 4. *The appearance of the morula.* FIG. 5. *An immature miracidium in an ovum on the twelfth day of development in water.* FIG. 6. *A mature and very active miracidium in an ovum after 30 days in moist feces.* FIG. 7. *Position taken by the miracidium immediately prior to hatching.* FIG. 8. *A miracidium in the position taken when swimming in water.* FIG. 9. *A 12-hour sporocyst.* FIG. 10. *A 48-hour sporocyst.* FIG. 11. *A sporocyst from the pulmonary sac of*

Sinitsin did not observe miracidia in the eggs before the thirty-third day and he notes that the cysts with which he worked had no exit via the bile system. These facts indicate that he was using eggs that had been held at body temperature for an abnormally long time and thus they cannot be considered as normal eggs of *F. magna*.

The development of the eggs in water or feces is not synchronous. At a mean temperature of 24° C. (maximum 27° C., minimum 21° C.) the majority of eggs in a water culture reach the morula stage in ten days. In one culture under three inches of water in a beaker, at the above-mentioned temperature and with the water changed daily, hatching commenced on the twenty-ninth day. These continued to hatch in numbers until the forty-fifth day.

Freshly evacuated eggs in the one-celled stage do not develop further during storage at a temperature of 2 to 5° C. Continual storage at this temperature up to at least a period of nine months, does not retard subsequent development. If the eggs at a late stage of development are frozen in water, they are promptly destroyed, although the miracidium in fully developed eggs may be slightly active for some hours after thawing. This activity has been observed in eggs after they have been frozen in water for 12-, 24- and 36-hr. periods, although the epithelial cells are in all cases badly damaged and the miracidium is incapable of swimming when artificially released from the damaged shell. Ova in the one-cell to the 16-cell stage are not readily affected by freezing in water or moist feces. A mixed lot of eggs was frozen at −5° C. in water and in feces for periods up to six weeks. Almost all the eggs which were at a stage less developed than the morula, were not affected, and developed normally when removed to laboratory temperatures. More detailed studies on the effect of cold had to be postponed owing to a seasonal difference in the rate of development.

The greatest factor in stimulating rapid development is temperature. Although development has not been studied under many different, controlled temperatures, it seems certain that the optimum is between 24 and 28° C.

On several occasions undried feces, which had been protected by vegetation and which contained eggs with active miracidia, have been found in the field. These eggs hatched normally and have been used in tests of possible intermediate hosts. Studies were subsequently made of this unusual development, using feces of *Cervus canadensis* which, when fresh, had a moisture content of 76-80%. Lots of fresh feces were placed in chambers and the moisture estimated by weighing portions, baking them for three hours at 56° C. and then reweighing. The samples at this time were hard and dry in appearance and to the touch. One lot, approximately 200 gm. in weight, was placed in a "moist-chamber" dish at 24° C., and the moisture gradually reduced by removing the water of condensation from the cover and sides daily. Some of the contained eggs were ready to hatch on the thirty-sixth day, when the moisture content of the feces was 61%. Other samples were prepared for observation by placing smaller amounts of fresh feces in three-inch glass-stoppered specimen jars and the moisture was reduced to a different degree

in each lot by exposing them to a dry atmosphere for various periods. After the jars had been sealed for 11 days the percentage of moisture by weight was calculated. No. 1 contained 76% moisture while Nos. 2–6, contained 73, 72, 66, 72 and 60%, respectively. In subsequent examination there appeared to be no difference in development, and on the thirty-fifth day after collection all the feces in each lot were sedimented and the eggs removed. In each lot about 40% contained active miracidia, 50% were in the morula stage and the remaining 10% were either destroyed or undeveloped. As this work was conducted in the autumn when other routine cultures were, for some unexplained reason, developing very slowly, further investigation was discontinued.

The above observations merely demonstrate that feces which fall into shaded places must be considered as possible media for viable eggs which can hatch during the course of a rainstorm or a flood. It is very probable that snails migrating from their habitat during a rainstorm are infested by miracidia which have suddenly hatched after having developed in feces.

The eggs are rapidly destroyed by complete drying, or by the reduction of moisture to a point below 50%. From these findings it is certain that eggs evacuated in feces and subsequently exposed to the full rays of the sun, will all be destroyed in less than three days.

Hatching

Eggs hatch sporadically, the phenomenon not being affected by light. Although most swarms of miracidia have emerged from the cultures at night time, hatching has frequently taken place under a strong artificial light. The results of attempts to determine the effects of darkness and light have been contradictory and the writer is convinced that the light factor plays little or no part in the process.

Attempts were made to stimulate hatching by controlling atmospheric pressure in a glass-topped receptacle. The eggs were placed in staining dishes such as are used for routine collection of miracidia and were carefully watched for periods ranging from one-half hour to one hour while under pressures varying from 25 to 33 in. No hatching was noted at any of these pressures or during any change in pressure, although nearly all the ova hatched normally following the experiments. These tests were conducted at various times of the day and evening.

Sudden changes in temperature will often accelerate hatching. Eggs taken from the refrigerator at 3° C. will frequently hatch in less than an hour in the laboratory at 22-24° C. This method is, however, far from infallible. Conversely, eggs will occasionally hatch on being moved from the laboratory to the refrigerator. One other factor controlling hatching is the aeration of water cultures. Eggs in stagnant culture dishes will remain unhatched for periods ranging from one to five months but when placed in fresh tap water or aerated water from aquaria, will soon hatch normally.

It is apparent that the phenomenon of hatching is controlled not by one, but by several factors, the temperature and aeration of the water playing the greatest parts.

In hatching, the miracidium straightens its head and pushes vigorously against the mucoid plug, with cilia lashing rapidly. It then attempts to straighten its whole body and, if successful, the operculum springs off or turns up as if on a hinge, the mucoid plug flows out and ruptures the vitelline membrane. The miracidium follows rapidly and swims away. One other phenomenon occasionally observed is hatching posterior end first: the miracidium turns completely around and, instead of pushing the plug with its anterior extremity, applies its exertions to the anti-opercular end. The operculum opens as before and the miracidium emerges, tail first, very much more slowly than it does by the more normal method because of the posteriorly directed ciliae. At the time the miracidium has emerged in this way as far as its head the body is stretched to a length approximately three times that of the egg.

The anti-opercular pore appears to play a part in the process of hatching by helping to offset the low pressure in the eggs caused by the exit of the mucoid plug, vitelline membrane and miracidium.

Snails eat eggs of *F. magna* very readily and it was thought probable that they become infested in this way. However, six snails placed in a culture from which miracidia were ready to emerge later evacuated eggs which were all unhatched. Since this simple test, embryonated eggs have been observed in snail feces upon many occasions and it is improbable that hatching is accelerated by the ingestion of the egg by the snail. Owing to the fact that the alimentary tract of the snail is very active and that evacuation of waste ingesta takes place soon after the food is swallowed, it is unlikely that infestation can result from this habit.

The Miracidium and Its Attack upon the Snail Hosts

Upon hatching, the miracidium swims in the surrounding water at a speed not approaching that of the miracidium of *F. hepatica*. Its speed has been estimated at 4 mm. per sec. by observing numerous specimens swimming over a scale on the stage of a dissecting microscope.

The miracidium can readily be studied in an egg albumen mount, but the portion of egg albumen on the slide should be allowed to dry around its circumference before placing the miracidium in the centre and applying a cover slip. This slight modification of Krull's method of using egg albumen as a mounting medium for larvae is more satisfactory for miracidia in that it prevents undue contraction of the body, owing to the increased viscosity.

In the normal swimming position the mean measurements of the miracidium are as follows: 0.211 mm. long by 0.032 mm. wide at a point immediately posterior to the eye spot. The head cilia are 10μ long; the cilia at mid-body

are 12μ and those at the posterior extremity are 16μ in length. The head collar is 0.023 mm. in diameter when in this position. The two flame cells appear to be 10μ long and are situated 81μ and 89μ from the anterior extremity.

Except in size the organism is very similar in general morphology to other fasciolinid miracidia.

The miracidia quickly die in water that has remained standing in the laboratory for some days, but will swim actively for 12-24 hr. in fresh tap water or in water from aerated aquaria. They do not appear to be attracted to either direct or diffused light; a strong microscope light does not affect them in any way.

The miracidia almost always swim in swarms and exhibit a similar appearance and the same activities as described for *Fasciolopsis buski* by Barlow.

The Attack upon the Intermediate Hosts

As described under snail hosts, the organisms do not show a distinct preference for their natural hosts, but readily attack several other gastropods. The strong chemotaxis described by Barlow (1925) for *Fasciolopsis buski* has not been observed. The miracidia will usually swim several times around a staining dish containing a suitable snail host before attempting to enter it. Some of them make futile attempts to penetrate the head and foot but the successful ones eventually find their way under the mantle fold and penetrate the posterior part of the pulmonary sac. For this reason the actual process of penetration into the host's tissues is rarely observed and some of the attempts to infest a snail with a single miracidium have failed owing to the uncertainty of penetration in spite of careful observation. From the study of the next stage in the cycle it appears that the penetration and the casting of the cilia and epithelial cells takes place as in related species.

The attack by miracidia does not appear to cause the snail any discomfort judging from its placid progress over the surface of the infection chamber even when many larvae are attempting to penetrate its tissues.

The Sporocyst and Rediae

Sinitsin (1933) describes a rather sensational method of parthenogenetic reproduction by the intramolluscan stages of *Fasciola* spp. He describes a phenomenon of "egg-laying" by the primary sporocyst or "euparthenita," the "eggs" developing into secondary sporocysts or secondary euparthenitae. These produce a great number of mother rediae or "migratory pseudoparthenitae" which migrate towards the liver substance, many of them being lost during this stage. The writer's findings do not agree with those of Sinitsin, and in the description of this more conventional cycle, the long accepted nomenclature of the larval forms is mainly used. The observations recorded here are those made upon infested snails kept at a temperature varying slightly from 24° C. Upon dissection of the snail, several hours after miracidial attack, the sporocyst or sporocysts is, or are, found in various

positions in the snail tissues. Twelve hours after penetration this form appears as a sub-circular body, from 55-70μ in diameter. Many of the miracidial characteristics are retained, but all the epithelial cells and cilia have been discarded. The flame cells of the excretory system are unchanged in size, activity or relative position; the mouth and rudimentary intestine are retained, the head end being invaginated into the "body". The eye spot is intact or may be divided into two or more fragments. The germinal cells are similar in size and appearance to those in the miracidium. This twelve-hour stage is only faintly motile, and it is probable that movement is accomplished by means of the body movements of the snail.

The position of the twelve-hour sporocyst in the host tissues varies and, owing to its extremely small size, it is difficult to determine accurately. If the snail is exposed to a large number of miracidia the resultant sporocysts are found in many positions at the end of 12 hr. They have been found in the lymph spaces around the albuminiparous glands, but are generally inside or on the borders of the pulmonary sac. After a further period of development most of these early forms disappear and at the end of the fourth day a maximum of two per snail have been found intact, even in heavily infested specimens. It is probable that the sporocysts that develop normally do so in the pulmonary sac, and owing to the resistance of the host's tissues, only one or two are able to survive even in this favorable position.

The growth of the sporocyst is very slow for the first 48 hr., but on the third day a distinct difference in size is seen. The germ cells show the greatest growth, one being very much larger than the others. On the fourth day the outline of the head end of the primary miracidium is obscured, but the flame cells are still unchanged and the eye spot is still present and, in some specimens, intact. At this stage an uncertainty existed regarding the mode of escape of the next form; in one specimen a number of germinal cells were seen clinging to the outline of the sporocyst in the region of the posterior pore. This phenomenon was at first thought to be the "egg-laying" as described for *Fasciola* spp. by Sinitsin, but examination of all the other specimens in the lot and also many subsequent ones failed to reveal a similar case. It was found possible to produce this phenomenon by squeezing the sporocyst and it is fairly certain that the specimen under discussion had been injured during dissection of the snail tissues.

On or about the sixth day after miracidial attack, the largest germinal mass has developed a distinct mouth and at this time makes its escape from the sporocyst. In most cases the rest of the germinal masses also emerge simultaneously, although they are comparatively poorly developed and do not show distinct mouth parts. These forms have been constantly traced by daily examinations of one or more specimens in each lot of infested snails, and have always developed into mother rediae. The existence of another generation of sporocysts has never been observed in either of the snail hosts in Canada. The premature emergence of the germinal masses seems to be the usual procedure. The mother rediae are thus found in very moderate

numbers in the liver tissue of the snail host, each one present being in a slightly different stage of development. In one lot of *F. parva* that were exposed to one miracidium per snail, and which were examined on the twelfth day, one was found to contain a single immature mother redia and no other forms. Another specimen contained two rediae and no other forms. Still another specimen contained two rediae approximately 0.200 mm. in length and ten smaller ones in which the mouth parts and enterons were indistinct. Measurements of three lots of mother rediae which were emerging from the sporocysts showed the largest to be 0.150 mm. by 0.05 mm. and the smallest 0.09 mm. by 0.06 mm.

The development of the mother rediae continues and between the twentieth and thirtieth day the daughter rediae are well formed.

A reliable difference noted between the mother and daughter rediae is the retention of the strong anterior collar in the former generation. In daughter rediae containing cercariae no indication of the collar can be seen. This indicates that the collar is formed by a simple fold in the wall which is taken up by the stretching effect of cercarial growth.

Mother rediae containing well developed daughter rediae have not been found larger than 0.87 mm. by 0.21 mm. In *S. palustris nuttalliana* the development is retarded and the rediae are very much smaller. Unfortunately the birth pore in this form has not been clearly seen, but it is apparently situated immediately caudad of the anterior collar. The excretory system of the mother redia is complicated and has not been worked out; the flame cells are numerous in the mid-body and the excretory pore is easily seen at the caudal extremity.

Daughter Rediae

When the daughter rediae emerge from their mother, they have well developed mouth parts and large enterons, the latter often reaching to the junction of the middle and posterior thirds of the body length. The germinal mass rapidly splits into eight to twelve round bodies which form the embryo cercariae. The daughter rediae grow rapidly from a size of approximately 0.3 mm. by 0.1 mm. to a maximum size of 3 mm. long by 0.33 mm. in maximum width.

In *F. parva* which appears to be a very suitable snail host for this parasite, no more than ten embryo cercariae have been found developing in a single redia. Under favorable conditions all the cercariae in a redia develop rapidly and a short time before emerging are in a similar stage of development. The continual production of daughter rediae by the successive development of the mothers ensures the emergence of cercariae over a long period.

The mature daughter redia is thus a large form. It does not retain the anterior collar and has smaller locomotor appendages and thinner walls than the former generation. The enteron is retained and contains a varying amount of ingesta depending upon the stage of development of the cercariae; this organ is frequently empty after the cercariae have emerged. This generation is only feebly motile throughout its life.

In the other snail host in Canada (*S. palustris nuttalliana*), a peculiar variation in the production of cercariae takes place. Instead of concurrent development of the embryos as in *F. parva*, they develop and mature successively. No more than three cercariae ready to emerge have been seen at one

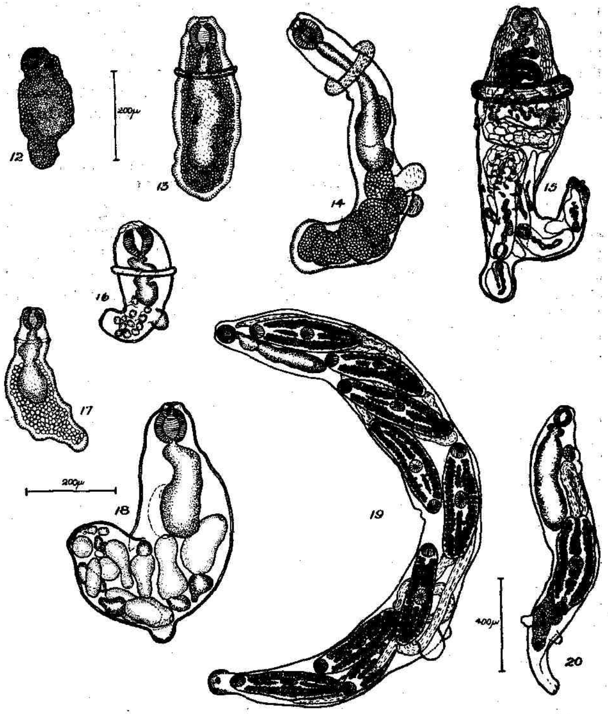

FIG. 12. *A mother redia free in the tissues of F. parva, a short time after its emergence from the sporocyst.* FIGS. 13-15. *Stages in the development of the mother redia, showing the characteristic anterior collar and posterior locomotor projections.* FIG. 16. *A single small specimen found in the liver of S. palustris nuttalliana on the fourteenth day of infestation.* FIG. 17. *An immature daughter redia shortly after emerging from a mother redia.* FIG. 18. *A typical daughter redia in the liver of F. parva, showing what has been found in this work to be a characteristic number of embryo cercariae.* FIG. 19. *An almost mature daughter redia in the liver of F. parva showing the concurrent development of cercariae. Compare this typical specimen with Fig. 20, which is the same generation in Stagnicola palustris nuttalliana.* FIG. 20. *A daughter redia from which most of the cercariae have emerged. This is typical of this second parthenogenetic generation in which the cercariae have developed successively*

time in a single redia. This finding has been constant throughout this work and appears to indicate that this snail is not a very suitable host. In order to show that this snail can and does act as a host throughout the intramolluscan cycle of the parasite, in spite of the fact that development is slower than in other snails, special observations were made upon the production of daughter rediae and cercariae. Results show that the numbers of daughter rediae do not vary from those found in the other species of snail. However, the production of cercariae is undoubtedly very much slower. This feature under natural conditions is consistent with the ecological finding that *S. palustris nuttalliana* lives under favorable conditions for longer periods than the other snail.

Experiment to Determine the Number of Daughter Rediae Produced Parthenogenetically from a Single Miracidium

Seven *S. palustris nuttalliana*, approximately ten days old, were each exposed to infestation by a single miracidium. They were then removed to a small tank to which was added clean vegetation but no extra source of calcium. These specimens retained the transparency of their shells and, by observation under low power magnification on the eleventh day, two were found to be infested. One specimen was killed on the twelfth day and five mother rediae were recovered. The other specimen was placed in a separate receptacle and observations upon the development of the parthenitae were continued. On the forty-ninth day several cercariae were seen to have emerged from the daughter rediae and to be moving rapidly in the badly damaged digestive gland of the snail. Many cercariae emerged on the fifty-third day and in two days 52 had encysted on the glass sides of the receptacle. The snail was then removed from the shell, and the digestive gland and adjacent tissues were fixed under a cover slip in warm 70% alcohol. The alcohol was then slowly replaced by glycerine, and the specimen was easily studied in detail. The forms present comprised a total of 21 large daughter rediae discharging cercariae, 16 small daughter redia containing an average of nine germinal masses and seven free cercariae in various stages of final development. To these must be added the 52 metacercariae obtained previously. The intramolluscan stages with their measurements are as follows:—

Large Daughter Rediae

(1) 1.33 mm. long by 0.211 mm. in maximum width. It contained one well developed cercaria 0.422 mm. in length, one partly developed, 0.165 mm. long, and two germinal masses approximately 50μ in diameter. Enteron 0.40 mm. long, oral sucker 72μ by 65μ in size.

(2) 1.05 mm. by 0.210 mm. Two cercariae, 0.351 mm. and 0.258 mm. in length. Enteron 0.390 mm. long, oral sucker 62μ in diameter.

(3) 1.17 mm. by 0.266 mm. Four cercariae, 0.446 mm., 0.435 mm., 0.280 mm. and 0.240 mm. in length. No germinal masses. Enteron 0.380 mm., oral sucker 60μ in diameter.

(4) 0.870 mm. by 0.203 mm. One cercaria 0.430 mm. long. Two germinal masses. Enteron 0.390 mm., oral sucker 60μ in diameter.
(5) 0.860 mm. by 0.195 mm. One cercaria 0.350 mm. long. Two large germinal masses. Enteron 0.395 mm.
(6) 0.664 mm. by 0.156 mm. One cercaria 0.275 mm. long. Seven germinal masses.
(7) 1.132 mm. by 0.234 mm. Cercariae 0.453 mm. by 0.172 mm. and 0.390 mm. by 0.125 mm. Germinal masses 0.177 mm. by 0.09 mm. and 0.082 mm. in diameter.
(8) 0.781 mm. by 0.150 mm. Enteron 0.318 mm. long. One cercaria 0.201 mm. Eight germinal masses.
(9) 0.790 mm. by 0.180 mm. Five cercariae, 0.273 mm., 0.203 mm., 0.160 mm., 0.160 mm. and 0.120 mm. Two small germinal masses.
(10) 0.870 mm. by 0.172 mm. Two cercariae 0.320 mm. and 0.340 mm.
(11) 0.780 mm. by 0.160 mm. One cercaria 0.205 mm. Three germinal masses.
(12) 1.05 mm. by 0.219 mm. Six cercariae 0.390 mm., 0.380 mm., 0.286 mm., 0.206 mm., 0.168 mm., and 0.160 mm. One germinal mass.
(13) 1.06 mm. by 0.250 mm. Three cercariae, 0.390 mm., 0.332 mm., and 0.312 mm.
(14) 1.00 mm. by 0.157 mm. One cercaria 0.320 mm.
(15) 0.890 mm. by 0.187 mm. Three cercariae 0.312 mm., 0.273 mm., and 0.250 mm. One small germinal mass.
(16) 1.18 mm. and 0.234 mm. One cercaria 0.280 mm. by 0.150 mm.
(17) 0.872 mm. by 0.158 mm. Two cercariae 0.370 mm. and 0.195 mm.
(18) 1.41 mm. by 0.235 mm. Six cercariae 0.400 mm., 0.397 mm., 0.395 mm., 0.375 mm., 0.203 mm., and 0.178 mm.
(19) 0.810 mm. by 0.195 mm. One cercaria 0.340 mm. Germinal masses ?
(20) 0.894 mm. by 0.155 mm. Two cercariae 0.235 mm., and 0.195 mm. Four germinal masses.
(21) 0.750 mm. by 0.162 mm. One cercaria 0.273 mm. Three (?) germinal masses.

In the above-mentioned rediae the enteron ranged in length from 0.318 mm. to 0.560 mm. The oral suckers had an average diameter of 60μ, the maximum size being 72μ by 62μ and the minimum 58μ in diameter.

Small Daughter Rediae containing cercarial germinal masses, the mean number of which was nine.

(1) 0.234 by 0.062 mm.
(2) 0.238 by 0.062 mm.
(3) 0.375 by 0.060 mm.
(4) 0.390 by 0.062 mm.
(5) 0.547 by 0.125 mm.
(6) 0.320 by 0.093 mm.
(7) 0.392 by 0.078 mm.
(8) 0.391 by 0.070 mm.
(9) 0.210 by 0.062 mm.
(10) 0.240 by 0.100 mm.
(11) 0.360 by 0.075 mm.
(12) 0.390 by 0.079 mm.
(13) 0.325 by 0.064 mm.
(14) 0.312 by 0.130 mm.
(15) 0.484 by 0.060 mm.
(16) 0.468 by 0.063 mm.

In the above-mentioned rediae the enteron varied greatly in size, the minimum length being 0.098 mm., and the maximum (No. 5) 0.290 mm. The oral suckers ranged in size from 56μ by 52μ to 45μ by 38μ.

In numbers, the above-mentioned daughter rediae do not differ from those found in *F. parva*. The more mature daughter rediae are, however, very much smaller owing to the fact that their cercariae have not developed at an equal rate. (Fig. 20.)

Cercariae

Of the nine cercariae present in the snail tissues, two encysted almost completely before they were fixed and two more only partly encysted owing to their immaturity. One which was completely, and two which were almost completely, coated with cystogenous material were successfully fixed and two immature ones were also fixed successfully.

The following measurements were taken:—

(1) Mature cercaria (capable of encysting). Body 0.386 mm. by 0.225 mm. Tail 0.910 mm. long by 0.064 mm. in median width.

(2) Immature cercaria. Body 0.296 mm. by 0.168 mm. Tail 0.880 mm. in length.

(3) Immature cercaria. Body 0.377 mm. by 0.202 mm. Tail curled.

(4) Completely encysted. Cyst 0.260 mm. by 0.220 mm. Tail 0.800 mm.

(5) Completely encysted. Cyst 0.255 mm. in diameter. Tail 0.937 mm.

(6) Almost mature. 0.317 mm. by 0.227 mm. Tail 0.590 mm. long.

(7) Almost mature. 0.305 mm. by 0.244 mm. Tail 0.670 mm. long.

The findings in this snail are consistent with observations made upon previous specimens of the same species which were not studied in detail, the parthenitae being used for individual study and dissection.

The Cercaria

The cercaria of *F. magna* is very similar in outward appearance and structure to that of other fasciolinid cercariae. The mean measurements of mature specimens, fixed in hot 70% alcohol, in a natural position are as follows:—

Body	0.300 – 0.321 mm. by 0.190 - 0.244 mm.
Tail	0.755 – 0.990 mm. by 0.045 - 0.057 mm.
Oral sucker	0.047 – 0.058 mm. in diameter (equat.).
Acetabulum	0.055 – 0.062 mm. in diameter.

Immature cercariae leave the daughter rediae as soon as the cystogenous glands are massed in the lateral fields (Pl. III, Fig. 5). At this stage the glands present the characteristic appearance described by Barlow as "the lower surface of a horse's hoof with the frog showing." It is this stage that has so often been taken from the snail's tissues and described as mature cercariae. Bovien (1931) pointed out this error in his study of *Cercaria Fasciolae hepaticae*.

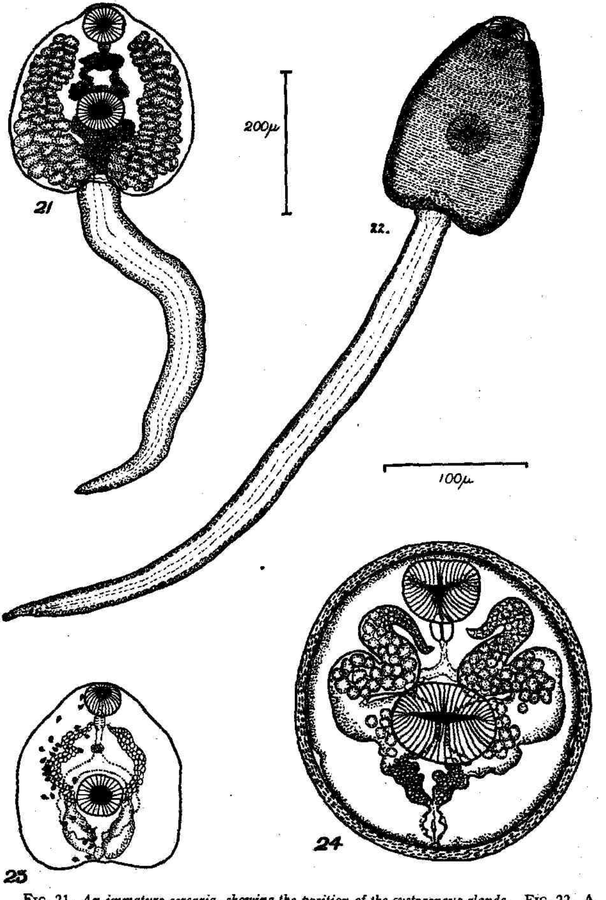

FIG. 21. *An immature cercaria, showing the position of the cystogenous glands.* FIG. 22. *A mature cercaria with an even coating of granules. This form is typical of free-swimming cercariae which are ready to encyst.* FIG. 23. *A partial flame-cell pattern as seen in a mature cercaria.* FIG. 24. *The metacercaria within its inner cyst.*

The cercariae of *F. magna* remain in the digestive gland of the snail for at least four days before emerging. When they do emerge they are mature, ready to encyst, and present a striking difference in appearance to those in the snail. They have acquired an even coating of cystogenous granules arranged in definite rows and which completely encircle the body: from 110 to 125 of these rows have been counted in individual specimens. The "hoof" appearance of the glands has been dissipated and the acetabulum is only seen as a faint outline under the coating. The granular coating is from 11μ to 13μ in thickness and is somewhat denser over the anterior part of the body where the rows are closer together (Pl. III, Fig. 6, and Fig. 22.)

The anterior extremity is the only part of the body which projects from this coating, and shows the characteristic spiny cuticula. Owing to the opacity of the granules the excretory system and the intestinal tract cannot be seen in mature specimens and a modified technique is necessary to remove the coating. This was done by pipetting the cercariae, as they emerged, into egg albumen, a cover slip then being closely applied. As they commenced to encyst, the slip was moved at approximately one-minute intervals until all the cystogenous material was removed. The specimen could then be retained in a live state in the medium for several hours.

The intestinal tract is essentially the same as that of *Fasciola* spp., the caeca being somewhat spiralled. Unfortunately it has not been possible to discern the entire solenocyte system of the excretory apparatus. However, sufficient flame cells were seen in one specimen (Fig. 23) to indicate that the formula conforms with that of the fundamental flame-cell pattern of the group (Faust, 1924). Minute spines are present on the anterior part of the body but do not appear to be present caudad of the acetabulum.

The tail is attached on the ventral surface at a point anterior to the posterior end of the body. The point of attachment in mature specimens is obscured by the granular coating. This organ is strongly muscular and is relatively larger than any other in the group.

In obtaining mature cercariae for study natural emergence was awaited and the specimens were immediately fixed in hot 70% alcohol. According to measurements of live specimens, this technique did not cause undue shrinkage. They were later dehydrated, cleared and then mounted in balsam. Staining was useless owing to the thickness of the granular coating which could not be satisfactorily cleared.

The Emergence of Cercariae

During the early stages of the infestation experiments, it was noted that all the cercariae emerged during the night. In order to check the consistency of this nocturnal phenomenon several infested snails were kept under observation all night. During the first night four snails were observed which had already produced many cercariae during previous nights and were apparently heavily infested. Two of these produced no cercariae. In one snail, nine emerged between 11:00 p.m. and 11:15 p.m., two being the greatest number

to appear simultaneously. Three cercariae emerged together from the other snail at 11:05 p.m. During the next night four more snails were observed but only one produced cercariae, three appearing between 1:00 a.m. and 1:30 a.m. Nine snails were observed at hourly intervals during another night, and forty cercariae appeared between 1:00 a.m. and 2:00 a.m., and five more during the next hour.

The above investigations were conducted under a weak artificial light, the snails being placed in individual petri dishes during the first two evenings and in one large one during the third night. In order to check this nocturnal habit further, 24 infested snails were placed in petri dishes and the encysted cercariae were counted at 5:00 p.m. and 8:30 a.m. for two days and nights. All the cercariae emerged at night between these hours. However, in one case that was not kept under observation a colleague reported the diurnal emergence of two cercariae, between 1:00 p.m. and 5:00 p.m. In two other cases the great activity of the tails which were still attached to the cysts at 8:30 a.m. indicated that the emergence had been more recent than six hours, although activity frequently lasts for three hours. An attempt was made to induce cercariae to emerge diurnally. All sources of light were removed from six dishes containing 12 snails during the daytime and a strong artificial light was applied at night. This was continued for three days and nights but in each case only nocturnal emergence occurred.

Depths at which Cercariae Encyst

Eight snails (*F. parva*) which commenced to discharge cercariae on the fifty-sixth day were placed in a standard small aquarium jar in 15 cm. of water. Vegetation was removed with the exception of a few pieces of grass and a piece of lettuce. One side of the jar was directed towards a window, two sides were covered with cardboard and one was exposed to dull light in the room. The sources of light were street lamps outside of the building and were of low intensity. Subsequent examination did not show a preference for the exposed sides, the greatest number of cercariae having encysted on a covered side adjacent to the food supply of the snails. At the end of 48 hr., 1004 encysted metacercariae were counted on the glass sides at the following depths below the surface of the water:—

1st centimetre	314	7th centimetre	22
2nd centimetre	316	8th centimetre	3
3rd centimetre	158	9th centimetre	4
4th centimetre	89	10th centimetre	1
5th centimetre	60	Below	0
6th centimetre	37	On the bottom	0

In a "moist-chamber" dish, in which were kept several snails of the same species as before, cysts were counted at the following depths:—

0 – 1 cm.—156 cysts.	2 – 3 cm.—140 cysts.
1 – 2 cm.—200 cysts.	3 – 4 cm.— 95 cysts.

On an area of the bottom, equal to the area of the wall upon which the above were counted, were 14 cysts. No cyst has ever been observed on the surface of the water, but they have been frequently found on the outer surface of the shell of the snail host. Although there was an abundance of sedge leaves, grass, algae and some lettuce in this and one other dish in which many cercariae emerged, all of them encysted on the glass. In order to obtain encysted forms on vegetation (Pl. III, Fig. 7), it was necessary to cover completely the inner surface of the glass sides of a vessel. Lettuce leaves were used for this purpose.

These findings indicate that the encysting habits are peculiar in that they are almost always nocturnal and that the cercariae do not encyst as readily on grass as do *Cercariae Fasciolae hepaticae*. This, and the complete absence of cysts on the surface of the water, show that infestation of the final host does not take place through drinking but rather by the ingestion of coarse vegetation.

The Metacercaria

The outer cyst of the metacercaria of *F. magna* is sub-circular in outline, 0.248 mm. to 0.275 mm. in diameter. These measurements do not include the adhesive substance around the cyst which projects from the outline for a distance of 12μ to 40μ. From a lateral view, the cysts are helmet-shaped, from 0.120 mm. to 0.130 mm. high. They are of a dark gold color when fresh, but with age become light yellow. The surface is coarsely reticular over the dome, but the side in contact with the object upon which they have encysted is smooth and the wall composed of a thin transparent adhesive substance containing few cells. The inner cyst is somewhat discus-shaped, the ventral surface concave to a depth of 30μ, and the dorsal surface convex to a similar degree. The measurements are, diameter 0.214 to 0.235 mm., maximum thickness 90 to 95μ. The hard inner cyst wall is 5 to 7μ in thickness, smooth in outline and in close contact with the metacercaria. The larva cannot be dissected intact from this cyst. The encysted metacercariae are unable to infest the definitive host if their outer cysts are removed. This was demonstrated by feeding twenty denuded inner cysts to each of two rabbits. As a control two other rabbits were fed with whole cysts at the same time. Post-mortem examinations on the twenty-eighth day revealed that the denuded inner cysts had not caused an infestation, but in each of the other rabbits, one marita was found. Owing to the findings of other workers, that metacercariae of related trematodes are unable to withstand digestion without an intact outer cyst, this fact was accepted without further investigation.

The oral sucker, acetabulum and excretory ducts conform to the general outline of related species. The excretory granules are coarse, and obscure the outline of the intestinal caeca.

Resistance of the Metacercaria to Partial Desiccation

The normally encysted metacercariae are very resistant to partial desiccation. One experimental jar, used previously for the investigation upon the depths of encysting, was retained for observations upon the effect of desiccation. The vegetation was removed and the water was poured off to a depth of 5 cm. All the encysted cercariae were then above the water line and the jar was left in the aquarium room at a mean temperature of 24° C. At bi-weekly intervals several metacercariae were removed and examined and at the end of sixty days, only a small percentage had been destroyed. At this time a rabbit was successfully infested with a marita from these metacercariae. On the ninety-second day the jar was removed to another room and at the end of 24 hr. all the larvae were found to be destroyed by desiccation.

At the time of removal from the aquarium room only half of the water below the cysts had evaporated, thus indicating a high relative humidity in this room. The relative humidity of the second room was then recorded and found to be 20%, this low degree being brought about by the steam heating appliances.

From this observation, it is apparent that the larva is not destroyed by desiccation while a certain degree of humidity is present in the atmosphere. It is generally agreed that the metacercarial stage of fasciolinid trematoda is readily destroyed by desiccation, and it has hitherto been believed that the larvae must remain in very damp surroundings if they are to survive. However, in spite of the fact that sheep have been experimentally infested by feeding "cured" hay, no attempt has previously been made to estimate the degree of relative humidity at which the larvae are destroyed. In order to clarify this point, a set of containers was prepared in which the relative humidity was controlled over solutions of sulphuric acid. The surfaces of the acid solutions were broken by coarse washed and baked sand, and the metacercariae, on glass, were suspended in the jars above the solutions. In order to eliminate any danger of injury to cysts, an aquarium jar which contained many newly encysted larvae was cut up into suitable pieces which were not allowed to become dry. The pieces of glass upon which numerous cercariae were encysted were placed in the test chambers immediately after the excess moisture was removed and the surfaces appeared to be dry. The cysts on some pieces were immediately examined in order to control this method of preliminary drying and all were found to be alive. In determining whether the larvae were alive or dead, the outer cyst was carefully dissected away and the outline of the inner cyst then examined. If this was normal in appearance and if the excretory tubules of the larva were in place and the

flame cells were active, it was considered to be alive. Dead metacercariae were easily identified by their partially collapsed inner cyst, the displaced excretory tubules and an absence of movement. The results of this biophysical experiment are shown in Table I.

TABLE I

RELATIVE HUMIDITY TESTS

Relative humidity, %	Hours tested	No. of cysts examined	No. of cysts dead	No. of cysts alive
15	24	20	20	0
15	65	26	26	0
20	24	11	7	4
20	65	39	39	0
25	24	25	24	1
25	65	36	35	1 (?)
30	24	10	5	5
30	65	35	34	1 (?)
40	24	12	9	3
40	65	37	31	6
50	24	11	4	7
50	65	40	31	9
60	24	18	14	4
60	62	33	28	5
70	24	14	3	11
70	65	19	11	8
70	120	16	15	1
80	24	10	1	9
80	65	35	25	10
80	120	20	15	5
90	120	17	4	13
95	120	30	0	30
95	240	20	0	20
95	480	22	2	20
95	576	10	1	9

Lack of suitable material prevented an extension of this investigation, but the results in Table I indicate that a very high humidity is necessary to ensure survival of metacercariae encysted on a non-porous substance and exposed to air. However, those that encyst on vegetation may survive very much longer in air even at low relative humidities. The comparatively large amount of water retained by plants could probably be utilized to prevent lethal desiccation of the cysts.

In summarizing the above observations on the effect of drying on metacercariae it is apparent that they possess individual powers of resistance. It is probable that they are able to exist under natural conditions for a considerable time after the water under which they encysted has receded. Practical tests on hay from an endemic area are now being conducted and it is hoped that the results will demonstrate the advisability of drying the hay for longer periods in order to ensure a greater death rate of the encysted metacercariae. Rajcevic (1929) has shown that metacercariae of *F. hepatica* remained alive in stacked hay for 17 months (two summers and one winter), proving the viability by feeding tests on sheep. Hay, therefore, must be recognized as a probable source of infestations by *F. magna* as well as by *F. hepatica*.

Artificial Infestations in Rabbits, Sheep and Guinea Pigs

In the experiments summarized in Table II all the animals used were young healthy stock, born and raised in this Institute. The animals used for other experiments, and subsequently killed and examined, were considered as controls:

TABLE II

INFESTATION EXPERIMENTS

Hosts	Cysts	Period of encystment	Period, feeding–slaughter, days	No. of maritae	Size of maritae, mm.
Rabbits Nos.					
1	10	3 days	33	0	— (Peritoneal lesions present)
2	10	12 hr.	103	1	7×4
3	9	8 hr. (or less)	33	2	3.7×1.8
4	10	12-24 hr.	30	1	2.0×1.2 (in peritoneal cavity)
5	10	12-24 hr.	28	1	1.2×1.1
6	15	4-5 days	28	1	3×1
7	15	4-5 days	28	0	—
*Sheep					
1	15	2-3 days	52	1	5.5×2.7
2	75	4 days	Still living, no eggs at end of 77 days		
3	75	4-6 days	Still living, no eggs at end of 77 days		
4	72	4-6 days	Still living, no eggs at end of 77 days		
Guinea pigs					
1	10	4-5 days	41	0	—
2	25	30-31 days	63	1	8.8×4.5

* *See page 214. "Further notes on Ovis aries as a definitive host of F. magna."*

These records do not include those animals used for the preliminary viability tests of dried metacercariae. All the metacercariae used in the infestations were constantly under the surface of water until used, when they were administered in a gelatine capsule filled with bran and ground oats. In the case of the rabbits the capsules were broken and the contents chewed before being finally swallowed. It is improbable in these cases that all the metacercariae arrived in the stomach intact in their outer cysts.

Owing to the extremely slow rate of development to the mature adult stage, it has not yet been determined whether maritae are able to develop to an egg-laying stage in the rabbit. Rabbits which received metacercariae were still negative to feces examinations for eggs four months later. One calf, repeatedly fed with viable metacercariae over a period of 20 days, was not passing eggs when the weekly examination was last made at the end of the twenty-second week.

Development of the Adult

As shown in Table II the young maritae developed very slowly in the animals which were artificially infested. In rabbits they wander over the peritoneum of the abdominal cavity for a long time. In one instance (No. 5) one was found under the peritoneum of the abdominal wall on the twenty-eighth day.

Sinuous caseated tracks mark the route taken by the parasite in the cavity. These tracks often lead to the surface of the liver into which the route is easily traced. The parasite wanders about in the liver tissue until finally encapsulated by fibrous tissue. In laboratory animals no complete encapsulation has so far been observed, probably owing to the limited period available before slaughter. In *Cervus canadensis* the lesion caused by young forms has been studied and in no case has the parasite been found in an egg-producing stage unless the encapsulation was complete. Forms have been found which have attained a size of 4 by 2 cm., which were in complete fibrous cavities and were still sexually immature.

Specimens removed from fresh liver and placed in normal saline at 38° C. are extremely active, but are not able to progress through the solution, their activity being confined to strong muscular contractions and bending processes. This muscular activity, combined with their cuticular spines as described by Stiles and Sinitsin, explains their ability to migrate through dense animal tissues with comparative ease.

The genital primordia are present in the maritae at a very early age, and have been clearly seen in all young specimens listed in Table II. However, further development is very slow and the period required to reach sexual maturity is still unknown.*

The largest adult recorded during this work was 8.1 cm. long by 3.2 cm. in greatest width. This measurement was made upon a live specimen stretched out on the palm of the hand. All anatomical details conform to the description of this trematode by Stiles, and any attempt to enlarge upon this thorough study would be superfluous.

The Location and Lesion Produced in the Tissues of the Definitive Host

Although *F. magna* is repeatedly mentioned as occurring in the lungs of its definitive hosts, it is unlikely that this is a common location.* The writer has never found this parasite in the lungs of heavily infested wapiti or any other ruminants or rodents, although the lung tissue was always examined with care. Francis (1891) states that he found them only in the liver of cattle in Texas. Curtice (1897) reported three cases of pulmonary distomatosis due to flukes which he later stated to be *F. magna* and also cites three more cases which were recorded in the "Veterinary Review", 1882, by A. J. Murray. I am unable to find any other definite records of pulmonary fascioloidiasis and am inclined to consider the occurrence as reported as an abnormal migration due to its occurrence in an abnormal host.

F. hepatica and *F. gigantica* are not infrequently found in the lungs of *Bos taurus*, in most cases being encysted and immature.

**See page 214. "Further notes on Ovis aries as a definitive host of F. magna."*

Francis described the migration of the marita in the liver tissue of *Bos taurus*. The channels that he observed in the tissue were large: "they admit the little finger and seem to heal or fill up soon after, leaving a red scar." He described the subsequent cyst, stating that the wall becomes dense and tough and is usually coated with a grit-like substance. He thought they died in these cysts, amidst the black fluid in which he found myriads of eggs. He found a maximum of five eggs in the entire quantity of bile that he collected from one animal, but states that he was not positive that those were eggs of *F. magna*. These findings in regard to the exodus of eggs into the bile system in *Bos taurus* agree with the recent findings of Sinitsin (1930 and 1933). It is peculiar that all the workers who have studied these eggs appear to have obtained their material from the fluke cysts in the liver of cattle and not from the feces. In the absence of adequate cattle data I have to rely on the observations of Francis and Sinitsin in propounding a hypothesis that egg evacuation in fascioloidiasis of cattle is abnormal and in some cases is non-existent. In *Bos grunniens*, as mentioned elsewhere, this is true in at least one case.

In addition to the above data regarding the abnormal parasitic life of this trematode in *Bos* spp., are the observations made upon the peculiar black discoloration on the mesentery, peritoneum and in the lymph glands, which was described by Dinwiddie in *Bos taurus*, and which is constantly seen in *Bison bison* and on two occasions has been observed in *Bos grunniens*.

In *Cervus canadensis* this discoloration occurs only when a closed or non-functional fluke cyst is present in the liver (See Plate IV, Fig. 1). When normal cysts only are present, discoloration of the tissues other than slightly darkened lymph glands and occasional black specks throughout the liver tissue, has not been observed, although numerous specimens have been examined. In the case of *Bos grunniens*, when all the cysts were "closed" the tissue discoloration was excessive, the liver being dark in color and the peritoneum, lymph glands and mesentery all containing blackened areas. Unfortunately, the whole livers of only two *Odocoileus virginianus* have been examined; in both cases the infestation was light and the cysts were open and no pigmented areas were present.

Salomon (1932), reporting the record in *Cervus elaphus* in Germany, described the macroscopic appearance of the liver, stating that there appeared to be no tissue reaction beyond the confines of the distended bile ducts. In the annual report of the Conservation Department of New York State, U.S.A., for 1933, cases of *F. magna* infestation of the livers of *Odocoileus virginianus* are reported but no mention is made of undue tissue reaction. In conversation and correspondence with observers who have seen fascioloidiasis in deer, no observations of severe tissue reactions or undue discoloration have been reported. It therefore appears probable from these findings in Cervidae, that this parasite finds a normal host in members of the Cervidae and is able to continue its normal life, producing eggs which are evacuated into the alimentary canal through the bile ducts.

In Bovidae, however, it appears that the tissue reaction to the presence of the trematode is so great that the "fluke cyst" is completely closed off in the majority of cases from the bile duct system and the powers of the parasite to reproduce are thus destroyed.

In order to support this hypothesis the tissue reaction has been studied in three hosts which harbored flukes in lesions typical of their species. The first is *Odocoileus virginianus* which was infested with three large trematodes, measuring 8.2 by 2.8 cm., 7.2 by 3.4 cm., and 8.6 by 3.1 cm., when fixed under slight pressure; these were contained in a single cyst from which open ducts conveyed the eggs normally. The second is *Cervus canadensis*, which was infested with fifteen flukes. In this case the infestation was so heavy that one cyst was closed and consequently the hepatic lymph glands and liver tissue were pigmented. (Pl. IV, Fig. 1.) The third host is *Bison bison*, in which the liver was infested with four flukes in two cysts. Both cysts were closed and extensive pigmentation was present in the tissues adjacent to the liver. Hadwen (1916) states that in an infested liver of *Odocoileus columbianus* the ducts were found to be dilated into pockets, these containing more "inky" bile than those caused by *F. hepatica*. However, accompanying photographs show that these lesions of fascioloidiasis were normal cavities with comparatively little pigmentation, and support my findings in other Cervidae.

The descriptions of the lesions are made under the names "open cavity" and "true cyst". The former name implies a continued evacuation of eggs into the bile ducts and the latter indicates a lesion which prevents egg evacuation, by occlusion of intercepted bile ducts.

Histopathology of the Liver Lesion and its Bearing on the Epizoology of *Fascioloidiasis magna*

Open Cavities

In cross section the fibrous cavities present a striking difference in structure from the lesions of other diseases caused by parasites in tissue. The wall is composed of fairly loose layers of fibrous tissue, the older tissue being on the outer surface of the cavity in contact with the liver tissue. The layers are successively more recent, the inner surface being composed of a layer of fine collagen fibrils and fibroblasts. The fibrous wall includes a blood-vascular system, and small bile ducts penetrate into the cavity. The outer layer of the wall is thus sharply differentiated from the surrounding liver tissue and, except in the region of bile ducts, there is no intermingling of liver and fibrous tissue.

The ducts, which have been intercepted in their course by the formation of the cavity, are thus divided into afferent and efferent ducts for the benefit of the enclosed parasite. The former supply the cavity with bile and the latter carry away excess bile, vomitus of the trematode, and eggs. While even a single cross section of a normal cavity reveals several bile ducts actually entering the cavity, no blood vessels have been seen doing likewise. In all probability these vessels are merely diverted from their course and some of

them may take part in the newly organized blood-vascular system of the wall of the lesion. In normal open cavities the bile ducts continue to function in their new capacity and, except for a slight fibrosis of the wall of the efferent ducts, they appear to be unharmed. In older cavities containing large mature flukes, the efferent vessels are seen to be fibrosed to a greater extent, in some cases being completely occluded by excess fibrous tissue which surrounds a mass of black vomitus and eggs. In most cases however, evacuation of eggs and detritus by the bile system is continued for a considerable time by the other open ducts.

In sections of the lesions, the path of primary migration by the young marita can be seen as lines of fibrosis in the liver tissue which have no direct relation to the bile system. This observation agrees with the observations made by Francis upon the migration through the liver tissue of cattle and with my own observations upon the migration in laboratory animals. It is evident that the marita comes to rest in the liver tissue as soon as the host's tissue reaction enforces it, and the cavity is then formed. The young fibrous tissue which has followed the path of the migrating marita and which approximates the final location is included in the formation of the fibrous cavity. At first the fibrous wall is composed only of a few strands of fibrous tissue (Pl. V, Fig. 2), but the growth and activity of the parasite enlarges the cavity until a surrounding area containing small masses of fibrosis, blood vessels and bile ducts is included. The liver tissue is destroyed by pressure atrophy until some time after the cavity has reached its maximum size, when normal liver cells are again found adjoining the fibrous wall. The size of the cavity varies greatly according to the size of the parasite or parasites, or according to the number. The largest open cavity observed was roughly spherical in outline and 4 cm. in diameter and contained three trematodes, the maximum number seen. The fibrous wall varies between 1.00 and 1.75 mm. in thickness.

The number of efferent and afferent bile ducts varies according to the size of the cavity. The trematode or trematodes lie coiled in the cavity, the ventral surface nearly always being in contact with the fibrous wall; this position is probably adopted to enable the acetabulum to fulfil its function. A comparatively small number of eggs is present in the cavity; these are mingled with a small quantity of bile and vomitus which appears microscopically as a black amorphous mass. In some cases masses of eggs and vomitus pigment are present in the wall, suggesting a fairly rapid growth of fibrous tissue during an egg-producing stage of the trematode's life in the cavity.

True or Closed Cysts

The above description covers briefly the general appearance of nearly all mature fluke cavities in the Cervidae. However, in heavy infestations in these animals there is often at least one closed cavity, which is a true adventitious cyst, filled with black fluid composed of bile, eggs, vomitus and often shreds of decomposed trematodes. This type of cyst is the typical lesion of

fascioloidiasis in the larger Bovidae, and indicates the important difference in the host's tissue reaction. It is this type of lesion that has been from time to time described as the ordinary lesion caused by *F. magna* in cattle. However, all previous workers appear to have merely described the external appearance of the liver and the appearance of the contained fluid and the incised cyst. From a comparative study it soon became certain that the closed cyst containing black fluid was the result of an attack by the parasite which had been overcome by the host's defence reactions. A study of this type of lesion shows an all-important difference in structure and an almost total obliteration of the ducts so essential for the normal evacuation of the trematode's eggs. This structure, in *Bos taurus*, can be explained by the well known fact that the fibrous tissue reaction to foreign bodies is particularly well marked in this animal. As stated by Dévé (1920) "..... on pourrait arguer que cette différence dans le mode de réaction des tissus au voisinage du parasite tient simplement à la différence du terrain, à l'espèce animale. Bien connue est, en effet, la tendance des tissue du bœuf à la réaction fibreuse et à la calcification des lésions parasitaires ou tuberculeuses."

The cyst is thick-walled, the fibrous tissue layers being more numerous and closer than those in the wall of a normal cavity. Both afferent and efferent bile ducts are totally occluded and are marked by tracts of fibrous tissue. The outline of the cyst is not well differentiated from the liver tissue owing to the extensive fibrosis of the bile ducts which involves the surrounding tissue. In some cases pigmentation is extensive, the pigment mass being very similar in microscopic appearance to the masses seen in cross sections of the caecal branches of the trematode. The chains of liver cells adjacent to the masses of fibrous tissue and pigmented areas are normal in appearance, and there is no evidence of recent inflammatory processes. No normal bile ducts have been found opening into a cyst which was filled with black bile and vomitus, and I am convinced that for only a short period during the trematode's early life in large Bovidae could they be found. In *Bos grunniens*, immature specimens of *F. magna* were found in closed cysts surrounded by the fluid which, of course, contained no eggs. It is probable that the trematode rarely reaches sexual maturity before being completely enclosed. That it does accomplish this sometimes is evidenced by the recording of a few eggs in the feces of *Bos taurus* by Sinitsin and the record of up to five eggs in the gall bladder of this host by Francis.

In addition to data obtained from past records in *Bos taurus* and the present examinations of a limited number of liver specimens from *Bison bison* and *Bos grunniens*, I am able to quote the work of Dr. R. Waddy, Inspector in the Health of Animals Branch. Dr. Waddy has made post-mortem examinations of some thousands of *Bison bison* and a few hybrids (domestic × bison) and mentions in his reports that extensive pigmentation occurs in the lymph glands and tissues adjacent to the liver in infested animals. This indicates a true cystic formation of the lesion. These reports also state that not more than four flukes have been found in one animal.

It is, therefore, apparent that the larger Bovidae, owing to their power of tissue regeneration, harbor the adult trematodes in true adventitious cysts. These animals are, therefore, unsuitable hosts for *F. magna*, preventing the completion of its normal processes of reproduction. It is altogether unlikely that the animals in which the efferent ducts are not completely closed can evacuate sufficient trematode eggs in their feces to offset the great hazards in the life cycle of the parasite. In support of this hypothesis one must consider that 1.85 eggs per gm. of feces are evacuated continually by a typical *Cervus* sp., infested with one trematode. This, in *Cervus canadensis,* means that each adult *F. magna* is producing at least six thousand eggs per day, all of which are evacuated normally. Even so, we do not encounter heavy infestations in *Cervus* spp. in most parts of North America, in spite of the known fact that several common snails can act as intermediate hosts. In cases of areas abnormally crowded with definitive hosts and rich in suitable snail life, we do see instances of super-infestations. I have examined an area, adjacent to the endemic locality to which reference has been made, that harbored similar snail life and over which a large herd of cattle ranged. There is no evidence of Cervidae and no case of fascioloidiasis has been observed during the past three years. On another large enclosed area only 13 miles from the above-mentioned ranch, in which several *Odocoileus hemionus* are enclosed, fascioloidiasis was present in the hybrid *B. taurus* × *B. bison*. Cervidae range freely over the area in which the Canadian bison are enclosed. The light infestations in these Bovidae can only be explained by reference to their feeding habits. Bison do not feed readily in low areas, visiting them only for the purpose of drinking, and retiring to higher land to obtain the preferred "prairie wool," to which cercariae would have no access.

It seems, therefore, very unlikely that *Bos taurus* and related Bovidae will ever be severely infested with *F. magna* unless Cervidae have access to their grazing lands. In other words, fascioloidiasis in large Bovidae occurs probably only in the presence of Cervidae. Unfortunately, in arriving at the above-mentioned conclusion the part played by the smaller Bovidae (*i.e., Ovis aries*) in propagating this disease cannot be stated.* However, experiments are in progress in an attempt to ascertain this point, but owing to the weaker power of tissue regeneration in *Ovis aries* than in *Bos taurus*, it is more than possible that these animals may play a role similar to the Cervidae. Sinitsin's hypothesis that sheep are not commonly infested, because *F. magna* can only complete its life cycle in areas in which there is an abundance of water for at least four months, is not supported by the present work.

Control of Fascioloidiasis Magna

The control of this parasitic disease differs in several respects from the measures employed for fascioliasis hepatica. Anthelmintic medication would probably be entirely ineffective owing to the location of the parasite in the

**See page 214.* *"Further notes on Ovis aries as a definitive host of F. magna."*

tissue of the host. Prophylactic measures must be employed following careful consideration of the definitive host involved and the character of the endemic area.

If the health of *Bos taurus* or other large Bovidae is to be protected, then all Cervidae should be eliminated from the grazing lands. This measure should reduce the danger of harmful infestations to a minimum. Care should be taken in importing Cervidae from North America, Italy or Germany. Such animals should not have access to areas inhabited by other Cervidae or Bovidae until examinations of feces have failed to reveal ova of *F. magna* for at least five months. As stated previously, it is unlikely that the importation of cattle from an infested area will cause an outbreak of fascioloidiasis in Bovidae in a previously free area, unless Cervidae are present.

In parks and other areas where deer are kept, or where there is an intermingling of these animals with Bovidae, destruction of the intermediate hosts is necessary. Much has been written about methods of snail destruction and copper sulphate is now in general use as the lethal agent. However, in the disease caused by *F. magna*, consideration must be given to the fact that three genera of Lymnaeidae are involved in the life cycle. One snail, *S. palustris nuttalliana*, shown in this paper to be an intermediate host in Canada, is ecologically very dissimilar to the other groups. This species inhabits permanent or semi-permanent, warm, stagnant bodies of water which are not favorable habitats for any other snails involved in the life cycles of liver flukes of ruminants. *Fossaria* spp. on the other hand are fairly consistent in their habitats, and methods so often described for snail destruction will be effective.

In Alberta, Canada, the area described under the heading of "Snail Hosts with Notes on their Ecology" was first treated, using copper sulphate for snail destruction. The stagnant bodies of water which harbored *S. palustris nuttalliana* were considered and a rough estimate was taken of the volume of water present. One part, by weight, of powdered copper sulphate was mixed with five parts of sand. After weighing but before mixing, the sand was slightly dampened to prevent undue blowing of the copper sulphate. This mixture was broadcast by hand over the swamps in amounts varying with the depth of surface water present. In swamps in which there was an average depth of water of 18 in., the mixture was applied at the rate of approximately 500 lb. per acre. This produced approximately a 1 in 430,000 solution in the swamps, which is theoretically sufficient to produce the desired lethal effect upon the snails, and to allow for loss by fixation of the salt by organic matter. In 1933 this method was completely successful in destroying this snail in several swamps in Alberta, and in May, 1934, no snails could be found in the treated waters.

One other method was tried in an attempt to destroy snails under the climatic conditions encountered in autumn on some Alberta prairie lands. Two swamps, which were known habitats for great numbers of *S. palustris nuttalliana* were selected. One was treated with the copper sulphate and sand

mixture at a rate of 250 lb. per acre when both were completely dry in early October. These swamps were dry until filled with snow water during the following spring. In May, 1934, both swamps were carefully examined and in the treated one only two young specimens of the snail could be found. In the untreated swamp, innumerable adult and juvenile specimens of the snail were present. This experiment, while being in no way conclusive, indicates a possible simple method of applying the lethal agent in western Canada.

In destroying *F. parva*, the above-mentioned mixture was broadcast over the described low, damp areas in May, when some free water was still present. The mixture was broadcast at the rate of approximately 250 lb. per acre, special attention being paid to water-filled animal tracks. Examinations made one week later did not reveal any living snails. It is probable that this method, or the one tried in October for destroying *S. palustris nuttalliana*, will be entirely satisfactory.

In recommending the destruction of *S. palustris nuttalliana* and *Pseudosuccinea columella* by applications of copper sulphate, special attention must be given to the fact that these snails, and closely related *Stagnicola* spp., frequently live in water inhabited by fish which may be killed by this salt. In these cases it would be advisable to rely upon the segregation of ruminants.

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It is with great pleasure that I acknowledge the help and co-operation extended to me by the National Parks Branch, Department of the Interior, Ottawa. Mr. J. B. Harkin, Commissioner of Parks, and Mr. Hoyes Lloyd, Chief of the Wild Life Division, with whose support this work was accomplished, have at all times extended the utmost co-operation and support on behalf of their Branch. Mr. A. G. Smith, Superintendent of Wainwright Park, provided excellent camping accommodation, help and equipment, and has at all times made my work pleasant and successful by his unselfish interest. His staff has, from time to time collected material, kept records and employed control measures, all of which work has been invaluable. The efficient methods employed by the Branch in preserving the health of their animals cannot be regarded too highly and augur well for the future of Canadian wild life and also domestic stock.

I also wish to express great appreciation of the co-operation of Mr. A. LaRocque of the Geological Survey, Department of Mines, and Dr. F. C. Baker, University of Illinois, who have so kindly identified the snails and have provided me with valuable records and much helpful advice.

Dr. E. A. Watson, Chief of the Pathological Division of the Health of Animals Branch, Department of Agriculture, under whom the preliminary survey work was commenced, kindly consented to the proposal that the work should be continued under this Institute, and I acknowledge his kind co-operation.

Further Notes on *Ovis aries* as a Definitive Host of *F. magna*.

Since the manuscript of this paper was submitted to the publishers, certain developments in the continued experiments on infestation of sheep have been noted. It is deemed necessary to include herewith some short notes on the development of the trematode in sheep and on its effect upon the host tissues.

Sheep No. 2 died on the 149th day after the ingestion of 75 encysted metacercariae. Although this animal was only parasitized by seven trematodes the liver tissue was severely damaged, and the animal exhibited marked emaciation. The largest trematode present was 3.2 cm. long by 1.9 cm. in maximum width, and was still sexually immature, although the uterus coils were partially developed. The other trematodes present were also sexually immature.

The tissue defence of this host was so weak that none of the parasites had become encapsulated; this resulted in the extensive tissue destruction and subsequent death, due to the unchecked migration of the parasite.

Sheep No. 4 died on the 150th day after a period of poor general condition but without marked cachexia. Post-mortem examination revealed the cause of death to be a diaphragmatic rupture. One lobe of the liver was parasitized by three trematodes, 3.8 cm. by 2.1 cm., 3.7 cm. by 1.7 cm., and 3.0 cm. by 2.2 cm. in size. The first two were almost sexually mature but were not producing ova. They were not encapsulated and were causing severe damage to the tissue of the lobe. The third specimen was partially encapsulated by fibrous tissue, and had reached sexual maturity, sixteen eggs being found in the gall bladder. Examination of the intestinal contents revealed the presence of five normal eggs. That this specimen had reached the stage of egg production very recently is evidenced by the facts that very few eggs were present in the bile and intestinal content, the uterine coils were not fully formed, and previous anti-mortem fecal examinations had not revealed ova. Thus *F. magna* may develop to sexual maturity in *Ovis aries* in a period of somewhat less than 150 days.

Sheep No. 3 is still living on the 166th day after ingestion of the metacercariae, but is cachectic and very weak. No ova can yet be found by fecal examination. Clinical studies are being made upon this animal.

The tissues from Sheep 2 and 4 are in course of preparation for histopathological study; however, from present macroscopic observations certain features are significant. *F. magna* is a very pathogenic parasite of this host owing to the fact that the tissues are unable successfully to combat its progress in the liver. This weak tissue reaction also enables the parasite to reach sexual maturity in lightly infested hosts and the ova are able to reach the alimentary canal by way of the bile ducts. *Ovis aries* must at present be regarded as a definitive host which enables the trematode to continue its life cycle, and thus it is able to play a role similar to that of the Cervidae.

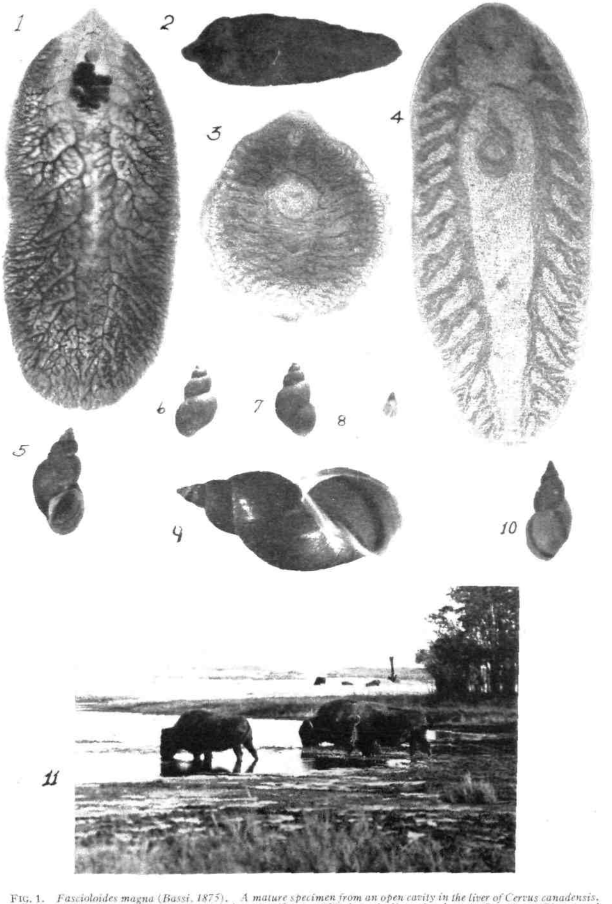


FIG. 1. *Fascioloides magna* (Bassi, 1875). *A mature specimen from an open cavity in the liver of Cervus canadensis. Actual length* = 6.2 *cm.* FIG. 2. *Fasciola hepatica* (Linn, 1758) *from the liver of a sheep in Canada.* (*Figs. 1 and 2 are on an equal scale to show the comparative size of these trematodes*). FIG. 3. *Young marita of F. magna from the liver of a rabbit 30 days after infestation* (×28.5). FIG. 4. *A marita of F. magna found in the peritoneal cavity of a rabbit 28 days after ingestion of encysted metacercariae* (×28.5). FIGS. 5, 6 and 7. *Fossaria parva* (*Lea*). FIG. 8. *F. parva* (*natural size*). FIG. 9. *Stagnicola palustris nuttalliana* (*Lea*). FIG. 10. *S. palustris nuttalliana* (*natural size*). FIG. 11. *A permanent habitat of Stagnicola palustris nuttalliana. The arrow points to a typical habitat of Fossaria parva in the background.* (*Photograph by Carsell, Wainwright, Alberta.*)

PLATE II

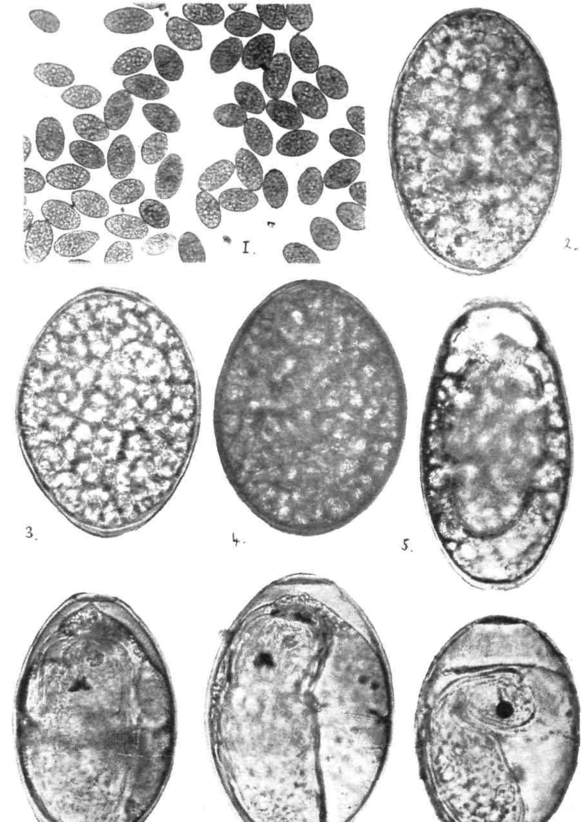


FIG. 1. *A group of ova screened from feces of Cervus canadensis, showing typical variations in shapes and sizes.* FIG. 2. *A newly evacuated ovum in the one-celled stage.* FIG. 3. *An ovum after three days' development.* FIG. 4. *An ovum in the 32-celled stage on the eighth day of development.* FIG. 5. *The morula stage.* FIGS. 5-8. *The development of the miracidium; Fig. 8 shows the typical position of a mature miracidium within the ovum.*

PLATE III

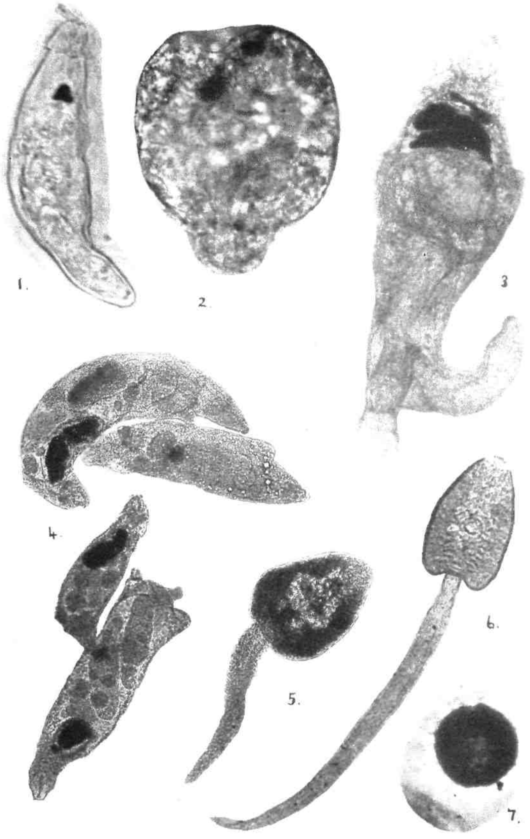
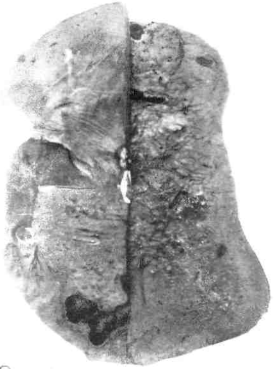


FIG. 1. *The miracidium with its anterior end extended (alive, in egg albumen).* FIG. 2. *A sporocyst from the pulmonary sac of F. parva, 12 hours after miracidial attack. Note the divided eye-spot.* FIG. 3. *A typical mature mother redia from F. parva.* FIG. 4. *A group of daughter rediae during the later stages of their development in the liver of F. parva.* FIG. 5. *An immature cercaria, free in the liver of the snail host. This shows the typical position of the cystogenous glands at this stage.* FIG. 6. *A mature cercaria, after having emerged naturally from the snail's tissues. The granular coating is clearly seen.* FIG. 7. *A metacercaria encysted on a lettuce leaf.*

1



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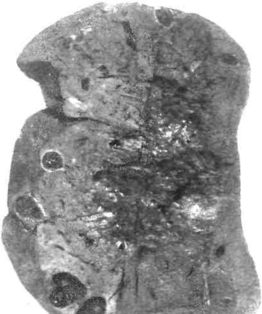

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4

FIG. 1. *An infested liver of Cervus canadensis.* FIGS. 2-3. *Sections of the same liver showing several normal cavities and a closed cyst which contained one live trematode.* FIG. 4. *A typical cavity in Odocoileus virginianus; this contained three large trematodes. Note the absence of pigmentation.*

PLATE V

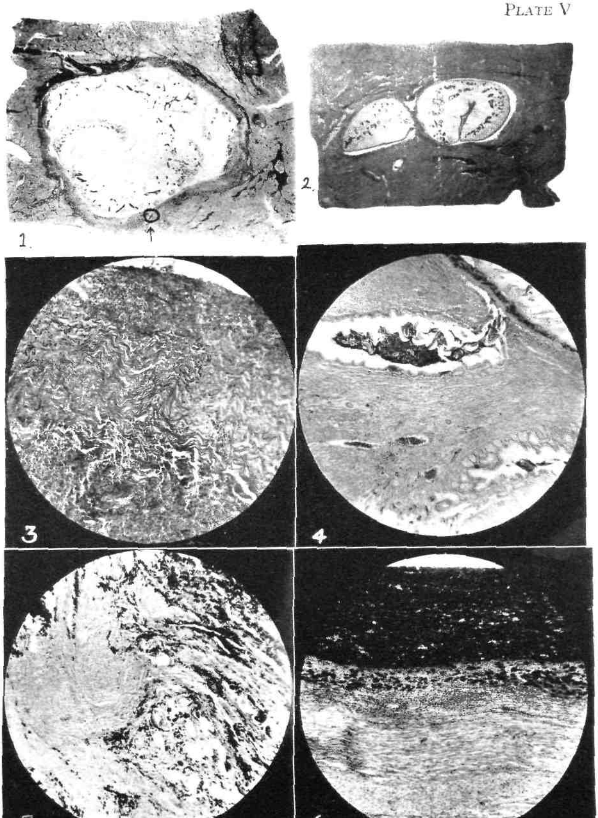

FIG. 1. *A section of a normal fluke cavity in the liver of Cervus canadensis. (The arrow and circle indicate the efferent bile duct as shown in Fig. 4.) Magnification* $\times 1 \cdot 74$. FIG. 2. *A young trematode in the liver of C. canadensis. The fibrous wall is being formed around the parasite. Magnification* $\times 2 \cdot 5$. FIG. 3. *A section through the wall of a normal cavity in the liver of O. virginianus. Note the normal liver tissue at the bottom and the successive layers of fibrous tissue. Magnification* $\times 54 \cdot 0$. FIG. 4. *The egress of vomitus and ova from the cavity through the open bile duct. (As Fig. 1.) Magnification* $\times 30 \cdot 5$. FIG. 5. *A section of the cyst wall in the liver of Bison bison, showing the inclusion of the blood-vascular system and the occlusion of the bile ducts.*

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ACTIA DIFFIDENS CURRAN, A PARASITE OF *PERONEA VARIANA* (FERNALD) IN CAPE BRETON, NOVA SCOTIA[1]

By M. L. Prebble[2]

Abstract

The immature stages of *Actia diffidens*, a tachinid parasite of the black-headed budworm, are described in detail, together with observations on the secondary integumental funnel, an ingrowth of the host body wall, within which the parasite maggots live in the later stages. In all cases observed, the integumental funnel was attached to a restricted area on either side of the mesothorax of the host larva. As the funnel is secondarily developed, its location can hardly be determined by the oviposition habits of the parent fly. Unfortunately, very little is known of the adult stage, and nothing of its mode of oviposition.

In an investigation of the natural control of the black-headed budworm, *Peronea variana* (Fernald), a tachinid parasite of interest was encountered. The presence of the older parasite maggots within the *Peronea* larvae was evidenced by a black circular disc on the mesothorax. The disc was the external indication of a secondary integumental funnel, formed from the body wall of the host larva and enclosing the parasite maggot, the posterior spiracles of which were applied against the central aperture of the disc. The parasite was not uncommon in 1930, and of considerable importance in 1931, but difficulty was encountered in rearing the adults. Adult flies were finally reared from overwintering puparia in the spring of 1932, after the *Peronea* outbreak had subsided. They were in 1933 identified by Dr. C. H. Curran as *Actia diffidens* Curran, a species established on material from Nova Scotia and New Brunswick (2). The allotype female was collected at St. Peters, Nova Scotia, in 1930, in the area infested by *Peronea variana*.

This paper embodies results from field studies in Cape Breton, N.S., supplemented by data from dissection, and microtome sections of preserved material. Although the data are incomplete, especially with respect to adult habits and the manner of oviposition, it seemed desirable to present them at this time, inasmuch as there is no likelihood that further material will be available in the near future.

Hosts of *Actia diffidens*, other than *Peronea variana*, are not known at present.

Description

Adult and Egg

Both sexes are described by Curran (2). The egg has not been observed, nor does the writer know where it is deposited. Many hundreds of *Peronea* larvae were examined during the two seasons, but only a very few dipterous eggs were noted (these were on full grown larvae). These eggs produced maggots which lived in the host larvae and pupae, without the formation of

[1] *This paper is on part of a project carried out by the Dominion Entomological Branch, the study of an outbreak of Peronea variana Fernald in the balsam and spruce forests of Nova Scotia, an account of which is now being prepared for publication.*

[2] *Junior Entomologist, Dominion Entomological Laboratory, Fredericton, N.B.*

an integumental funnel, so it is quite certain that they were of another species. Nor were egg shells found on *Peronea* larvae known to be parasitized by *Actia diffidens*. It seems quite improbable, therefore, that the eggs are laid externally on the host larvae, as are those of some other species of *Actia*.

First Instar (Figs. 4, 5 and 30)

The first-instar maggot ranges from .5 mm. to .7 mm. in length, is white, and tapers towards the extremities. Two pairs of sensory organs occur on the head, and are similar to those of the third instar; the anterior sensory organ has a diameter of about .006 mm.; the posterior sensory organ consists of two larger papillae and four or five smaller papillae. Anterior spiracles absent; posterior spiracles not evident on cleared skins under oil immersion lens, though a pair of minute tubes, presumably the dorsal tracheal trunks, were observed in the caudal segment; stigmatic chambers not observed on extremities of tubes, so it is doubtful if posterior spiracles are functional in the first instar.

The buccopharyngeal armature (Figs. 4 and 5) has a length of .07 mm. to .09 mm. The median tooth is broadly rounded on the dorsal side, nearly straight on the anterior margin, and produced into a short acute tip ventrally. Posteriorly the armature branches into two symmetrical arms, each with a dorsal and ventral wing; these wings are much smaller than those of other primary tachinid larvae. The cast-out buccopharyngeal armature is similar to that of young maggots, so there is no progressive sclerotization, such as is characteristic of some other tachinid species. The anterior third is less heavily sclerotized than the remainder of the armature. A small lance-like sclerite, the "lateral anterior plate" of Thompson (8) occurs on each side of the median tooth.

Spinules occur in 11 transverse bands, as follows: The first band is near the junction of the head and prothorax, and encircles the body; it consists of six or seven interrupted rows of very minute spinules, barely visible under the high power of the microscope; the spinules in the first few rows on the venter are larger than the others. The second transverse band consists of eight or nine interrupted rows of spinules, and encircles the body from the posterior region of the prothorax to about the middle of the mesothorax; the spinules in the anterior two or three rows are the heaviest, and are directed to the rear. The third band consists of about five rows of spinules, and encircles the body at the junction between the mesothorax and metathorax. The fourth band occurs at the junction of the metathorax and first abdominal segment; on the ventral side there are five or six rows of spinules (those of the first two rows being larger), but only one or two interrupted rows of spinules extend to the dorsum.

The next six bands (fifth to tenth, inclusive) occur at the margins between successive abdominal segments from the first to the seventh; they are practically confined to the venter, only a few small spinules occurring on the sides. Each band is broken into an anterior and posterior series, with a smooth space

between (see Fig. 30, A). The anterior series of each band consists of three or four rows of spinules, of which those in the first one or two rows point forward, and those in the following rows point to the rear. Usually the spinules of two adjacent rows in the anterior series are heavier than the rest, those in the first heavy row pointing forward, those in the next row pointing backwards. The posterior series of each band consists of three or four broken rows of small spinules, all of which point to the rear.

The eleventh band occurs at the junction between the seventh and eighth abdominal segments (Fig. 30, B). The band encircles the body, and consists, on the ventral side, of four or five rows of spinules (those of the anterior row are small, those of the following rows are much larger), but on the dorsal side there are only one or two rows of spinules. About midway along the lower side of the eighth abdominal segment there occur two or three irregular rows of spinules. Practically all spinules on the final segment point forward, the largest, those on the venter, being about .004 mm. long.

Second Instar (Figs. 6, 7, 19 and 31)

The second-instar maggot ranges from less than 1 mm. to about 3 mm. in length. The head is small, almost hemispherical, and the sensorial areas are similar to those of the third instar. Anterior spiracles were not observed, even under the high power of the compound microscope; their rudiments may be present in the hypoderm, but in all probability they are not functional till the second moult. The posterior spiracles are borne on the caudo-dorsal region of the final segment. Each spiracle (Fig. 19) consists of two minute circular openings, and has a maximum width of about .025 mm. The stigmatic chambers are short, of about the same diameter as the dorsal tracheal trunk, yellow, and granular in appearance. The posterior spiracles are typically separated by four or five times their width, though in some specimens they are much farther apart, and in others much closer together. No anal opening was observed. Presumably the hind gut has no connection with the exterior until the second moult.

The buccopharyngeal armature (Figs. 6 and 7) has a length of .12 mm. to .13 mm. The paired oral hooks have a long acute anterior tooth, a stouter posterior ventral tooth, and a caudo-dorsal extension above the point of articulation with the pharyngeal sclerite. The pharyngeal sclerite has an anterior bar-like extension which articulates with the oral hook of the same side, while posteriorly it bifurcates into a stout dorsal wing, and a ventral wing which is sclerotized only on the dorsal margin. The dorsal wing has a short anterior projection. The ventral trough of the pharynx is lightly sclerotized. The plate of the salivary duct lies somewhat below the level of the pharyngeal sclerites (Fig. 7).

Eleven transverse bands of spinules occur on the ventral region, near the margins between successive segments. Each transverse band, with the exception of the first, occupies the posterior region of the preceding segment, and the anterior region of the following segment. The first band occurs

between the larval head and the prothorax, the eleventh along the margin between the seventh and eighth abdominal segments. The first band consists of a single row of indistinct, backwardly directed spinules, and extends part way up the side of the larval head. The second, third and fourth bands consist of four interrupted rows of spinules, directed caudad; frequently these rows are reduced to three on the sides, and the spinules are noticeably smaller, extending about half-way up the sides. The fifth band (at the margin between the first and second abdominal segments) consists of three rows of spinules; on the venter there is a smooth region between the second and third row of spinules. All the spinules point to the rear. The sixth band is composed of a short anterior ventral row of very minute spinules, pointing forwards, followed by four rows of larger spinules which point backwards; there is a smooth region between the third and fourth rows; on the side there are two anterior rows of minute forwardly directed spinules. The seventh band consists of a short anterior row of minute, forwardly directed spinules, followed by three rows of larger spinules which point to the rear; there is a clear space between the third and fourth rows, and on the sides another short anterior band of forwardly directed spinules is present. The eighth band (Fig. 31, A) consists of three anterior rows of very minute, forwardly directed spinules, followed by three or four rows of larger spinules directed to the rear; the rows are broken toward the upper extremities, and the spinules are smaller. The ninth band is essentially similar to the eighth; and the tenth band is similar, except that four anterior rows of forwardly directed spinules are present instead of three. The eleventh band (Fig. 31, B) completely encircles the body between the seventh and eighth abdominal segments, and the spinules all point forward; in the ventral region there are two or three anterior rows of small spinules, followed by three or four rows of much larger spinules; on the sides and dorsum the spinules are smaller. A few small spinules occur about halfway between the heavy ventral spinules and the posterior extremity of the eighth segment.

The largest spinules in the anterior bands are about .003 mm. long, while the stout spinules at the base of the eighth abdominal segment are about .007 mm. long. They are shown enlarged in Fig. 31.

In some of the older second-stage maggots the first ten bands of spinules are difficult to detect, and in others they were not distinguished at all. The anterior bands of spinules were never detected on third-stage maggots, although the band around the margin between the seventh and eighth abdominal segments is well developed, and spinules are present in profusion about the posterior spiracles.

Third Instar (Figs. 8 to 18)

The third-instar maggot (Fig. 10) ranges from 3 mm. to over 5 mm. The larval head is small, almost hemispherical and bears two pairs of circular sensorial areas. The anterior pair are the larval antennae, or "anterior sense papillae" of Snodgrass (7); they are slightly elevated above the head

wall. The posterior pair are presumably the maxillary organs, or "posterior sense papillae" of Snodgrass. Seven or eight minute pits were noted in the circular papillae of some specimens, but were indistinguishable in others. The oral hooks lie in lateral pouches of the oral cavity, separated by an indistinct dorsal ridge.

The anterior spiracles are borne in depressions on the sides of the prothorax, near the posterior margin. Each spiracle (Fig. 12) is flattened in a vertical plane, and has five to eight orifices at the end of finger-like projections; the most common number of openings is six, but there often is asymmetry in specimens. The stigmatic chambers are yellow, and granular in appearance, and of smaller diameter than the dorsal tracheal trunks.

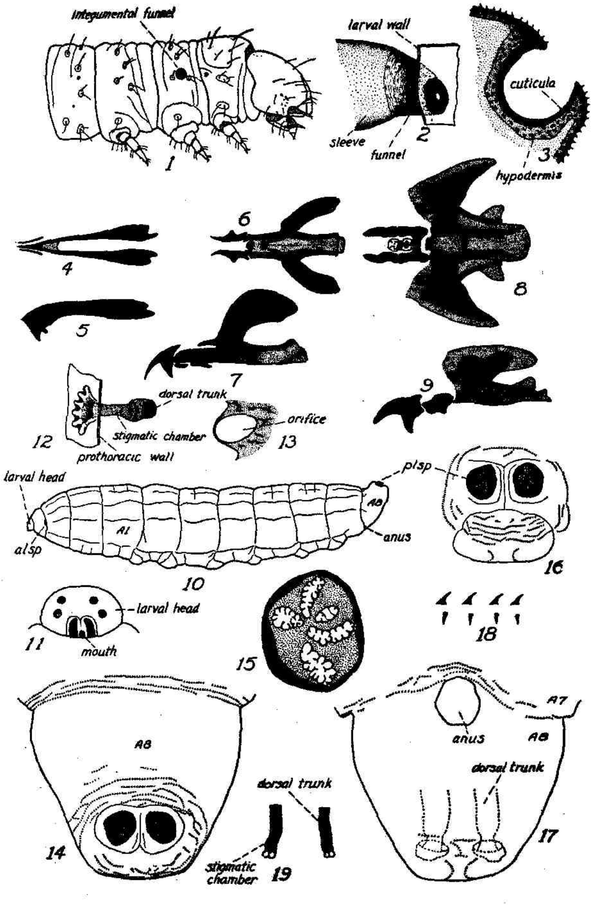
The posterior spiracles are located in a shallow depression on the tip of the eighth abdominal segment, which curves dorsally to fit snugly into the base of the integumental funnel. The stout cylindrical peritremes project above the contour of the segment, and have a diameter of .12 mm. to .17 mm.; they are separated by about half their diameter. Each spiracle has five oval to linear openings, surrounded by dark rims from which curved or forked processes are directed inwards. Usually the lateral stigmatic plate is considerably smaller than the other four.

The anus is situated on the ventral surface of the eighth abdominal segment, near the anterior margin.

The buccopharyngeal armature (Figs. 8 and 9) has a total length of about .34 mm. The oral hooks have a blunt anterior tooth and a sharper posterior ventral tooth; there is a concavity on the caudo-dorsal margin, into which the anterior ends of the lateral arms of the hypostomal sclerite fit. The hypostomal sclerite is typically H-shaped viewed from above, the transverse bridge connecting the lateral arms on the ventral side. The posterior portion of the buccopharyngeal armature consists of a lightly sclerotized ventral trough, supported on each side by the lateral pharyngeal sclerites, whose form is illustrated in Figs. 8 and 9.

Spinules occur only at the posterior end of the body, where they are arranged in two circular bands, the first near the junction between the seventh and eighth abdominal segments, and the second band about the posterior

FIG. 1. *Head and first four body segments of mature Peronea variana larva, showing location of secondary integumental funnel in side of mesothorax.* FIG. 2. *A portion of the mesothoracic wall, showing the basal portion of the funnel.* FIG. 3. *Transverse section through the anterior portion of the disc and funnel, showing continuity of cutaneous layers.* FIG. 4. *Dorsal view of the buccopharyngeal armature of the first-stage Actia maggot.* FIG. 5. *Lateral view of the same.* FIG. 6. *Dorsal view of the buccopharyngeal armature of the second-stage Actia maggot.* FIG. 7. *Lateral view of the same.* FIG. 8. *Dorsal view of the buccopharyngeal armature of the third-stage Actia maggot (lateral wings spread out somewhat from normal position).* FIG. 9. *Lateral view of the same.* FIG. 10. *Lateral view of the third-stage Actia maggot; alsp, anterior larval spiracle; plsp, posterior larval spiracle.* FIG. 11. *Ventral view of larval head, third-stage maggot (enlarged).* FIG. 12. *A portion from the prothoracic wall of the third-stage maggot, showing anterior spiracle and the stigmatic chamber (enlarged).* FIG. 13. *An orifice of the anterior spiracle of the third-stage maggot (very greatly magnified).* FIG. 14. *Dorsal view of tip of abdomen, third-stage maggot (enlarged).* FIG. 15. *View of right posterior spiracle (greatly enlarged).* FIG. 16. *Enlarged caudal view of tip of abdomen, showing spiracles, spinules, and sensory setae.* FIG. 17. *Ventral view of tip of abdomen, third-stage maggot.* FIG. 18. *Spinules of the third-stage maggot viewed from side (upper) and from above (lower).* FIG. 19. *Posterior spiracles and stigmatic chambers of second-stage maggot.*


integumental funnel
1
larval wall
sleeve
funnel
2
cuticula
hypodermis
3
4
5
6
7
8
9
dorsal trunk
stigmatic chamber
prothoracic wall
12
orifice
13
larval head
alsp
A1
A8
plsp
anus
10
16
larval head
mouth
11
15
18
A8
14
dorsal trunk
stigmatic chamber
19
A7
anus
A8
dorsal trunk
17

spiracles (Figs. 14, 16 and 17). The spinules are about .005 mm. in length, triangular when seen from above, but curved and sharp seen in side view (Fig. 18). As in preceding instars, the arrangement of the spinules varies somewhat, but the anterior band consists of three or four interrupted rows of spinules, which are fairly regular on the dorsum (Fig. 14), and more irregular on the venter (Fig. 17). Some of the spinules in front of the anus may be directed posteriorly, but nearly all other spinules in the anterior band point cephalad. The posterior band consists of about four irregularly broken rows of spinules encircling the truncate tip of the abdomen (Fig. 14); the spinules are more numerous above and below the spiracles, and rather sparse laterally. Nearly all the spinules in the posterior band point cephalad, that is, with the apices directed away from the posterior spiracles. The spinules below the spiracles are borne on an oval elevation (Fig. 16). On the same elevation there occur two minute sensory setae; these were not observed on the preceding instars.

Four longitudinal rows of creases extend from the second thoracic to the seventh abdominal segment (Fig. 10); these creases are also noticeable in the puparium. Six transverse ambulatory elevations of the body wall occur on the venter, between successive abdominal segments, the first occurring between the first and second abdominal segments, the last between the sixth and seventh segments. Less prominent elevations occur on the anterior segments.

The Puparium (Figs. 24 to 27)

The puparium ranges from 2.8 mm. to 4.5 mm. in length, and from 1.3 mm. to 2.0 mm. in width. Yellow at first, it soon turns brown. Eleven segments are distinguishable, the first being the prothorax; apparently, as in *Rhagoletis pomonella* Walsh (7), the larval head is involuted through the anterior opening. The anterior end is rounded, and is marked by horizontal and transverse creases which represent the lines of cleavage of the puparial cap. The horizontal line arches over the mouth opening (Fig. 25), thence back below the prothoracic spiracles to the anterior margin of the first abdominal segment, where it meets the transverse line which encircles the puparium. The transverse line is heavier on the dorsal half, and the dorsal half of the puparial cap is usually pushed off at emergence.

The anterior spiracles (Fig. 26) are flattened, inconspicuous, and consist usually of six lobes, though the number is variable as in the larva. The dorsal half of the prothorax, above the horizontal cleavage line, is marked by about seven faint creases which give it a rugose appearance. The mouth opening is marked by an oval scar at the centre of the prothorax (Fig. 25).

The posterior spiracles project slightly above the general contour (Figs. 24 and 27). The anus is visible as a circular scar at the anterior margin of the final segment, and from a narrow slit in the scar there projects inward a short stalk, the remnant of the larval proctodeum. There are two protuberant areas on the venter of the final segment, one bearing the anus, the other representing the elevated and spinulated region described for the third instar.

Abdominal spiracular openings could not be detected on overwintering puparia, or on puparia from which the flies had emerged. There is, however, on each side of the first abdominal segment, near the posterior margin, a circular opening (Fig. 24) through which the prothoracic cornicles of the pupa (Fig. 23) are thrust.

The longitudinal creases of the third-instar maggot appear on the puparium, as do also the remnants of the ambulatory elevations. The latter have the form of narrow rugose areas at the margins between successive abdominal segments, between the first and second segments, to the sixth and seventh segments.

The surface of the puparium is faintly rugose transversely, and this is more pronounced on the final segment. The larval spinules of the final segment are visible under high magnifications.

Prepupa

Within puparia which were opened while the insect was still in the pupal stage, was found a fine transparent membrane, lying snugly against, but separable from, the puparial wall. The cast-out buccopharyngeal armature of the third instar, the linings of the dorsal tracheal trunks, and the proctodeal lining, lie on the inner surface of this membrane, that is on the surface exposed when viewed in a cut-open puparium. This membrane is similar to the prepupal cuticula of *Rhagoletis pomonella*, concerning which Snodgrass writes as follows: "Now, from reading most descriptions of fly metamorphoses, we should expect to find a pupa within the puparium. But the object disclosed in the puparial capsule of the apple maggot at this stage has no character suggesting a pupa . . . . The new creature, moreover, has a cuticle distinctly its own . . . . In fact, it is evident that the maggot of the third instar has changed by a puparial molt, not to a pupa, but to a *fourth* instar larva, an intra-puparial, prepupal larval stage, complete in all respects except for the retention of the stomodeal, proctodeal and tracheal linings of the third larval instar . . . . The larva of *Rhagoletis* remains in this post-puparial instar a varying length of time, but usually not beyond the end of 48 hours from the time it entered the ground. Then begins another molt which ends in the complete shedding of the papillated cuticle and in the disclosure of the true pupa."

Though no puparia of *Actia diffidens* were opened before the pupa was formed, that is, while the insect was in a prepupal stage, it appears quite conclusive, from the presence of this membrane and its relation to the cast-off chitinous parts within it, that there is a prepupal stage, corresponding in its essential features, to that which occurs in *Rhagoletis*.

Pupa

A small number of puparia were cut open in October, about two months after being formed, and the pupae were in the stage of development illustrated in Figs. 20 to 22. The insect probably overwinters in this, the phanerocephalic pupal stage. The eyes were not distinguished, nor were the abdominal segmentation, or the abdominal spiracles, evident on uncleared specimens.

The anterior pupal spiracles (Fig. 23) consist of a pillar-like prothoracic cornicle, borne on a high mound of the body wall, and a two-lobed inner spiracle, borne on a smaller elevation. The prothoracic cornicle has a simple orifice, which is thrust into the air through the opening on the first abdominal segment of the puparium. The inner spiracle has two lobes, each of which carries over twenty small oval orifices; it communicates with the interior of the puparial chamber. The yellow, granular, stigmatic chamber of the pupal spiracle is separated into two parts, a large ovoid chamber beneath the prothoracic cornicle, and a two-lobed chamber beneath the inner spiracle, which communicates posteriorly with the dorsal tracheal trunk.

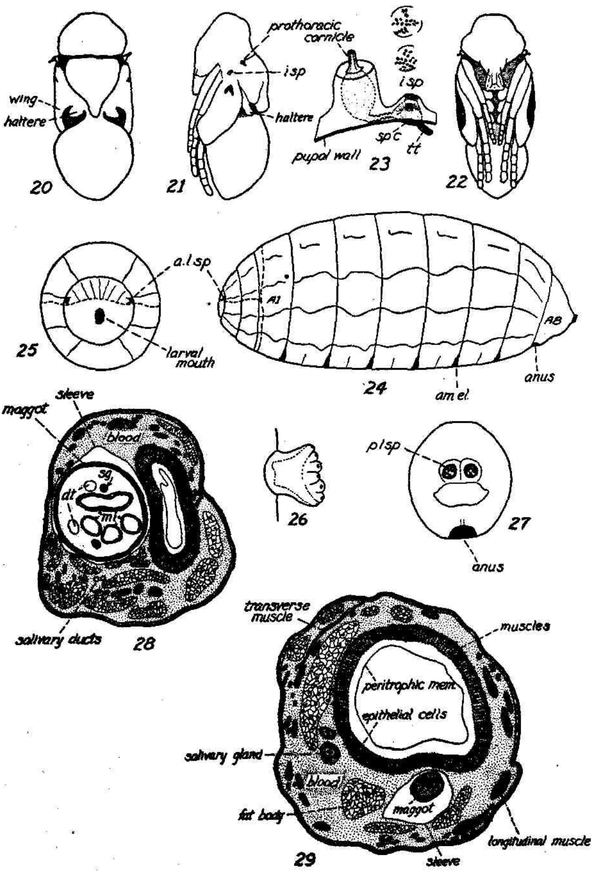
Life History

Adults emerged the last few days of May and the first of June at the Fredericton laboratory. Probably emergence is later in the field. Adults were taken in flight in Nova Scotia during the middle part of June.

Parasite maggots were recovered in small numbers from *Peronea* larvae which had been isolated in the third stage, and in larger numbers from *Peronea* larvae isolated in the fourth and fifth stages. Judging by the occurrence of the host larval stages in Cape Breton, parasitism commences late in June, and is heaviest the first half of July. The full-grown maggots issue from the dead hosts from the middle of July to the middle of August, drop to the forest floor and form their puparia.

The results obtained from dissections of host larvae are as follows: *(a) Peronea* larvae in which no funnel was developed: First- and second-stage *Actia* maggots were found in fourth- and fifth-stage host larvae, living *freely* in the body cavity from the prothorax to the tenth abdominal segment; *(b) Peronea* larvae in which the funnel was developed: Second-stage *Actia* maggots were commonly found in funnels, and all third-stage maggots were dissected from funnels. When the enclosed maggot was in the third stage, the buccopharyngeal armature of the second stage was always lodged in the base of the funnel. In a very few instances the buccopharyngeal armature of the first stage was also found in the base of the funnel, showing that the funnel had developed before the first moult; but usually the armature of

FIG. 20. *Dorsal view of overwintering pupa.* FIG. 21. *Lateral view of the same; isp, inner spiracle.* FIG. 22. *Ventral view of the same.* FIG. 23. *A portion of the pupal wall, showing prothoracic cornicle and inner spiracle, isp (enlarged above), stigmatic chamber, spc, and dorsal tracheal trunk, t.t. (enlarged).* FIG. 24. *Lateral view of puparium (enlarged); alsp, anterior larval spiracle, am, el, remains of larval ambulatory elevations.* FIG. 25. *Enlarged anterior view of prothorax and mesothorax of puparium.* FIG. 26. *Anterior view of anterior larval spiracle on puparium (greatly enlarged).* FIG. 27. *Enlarged posterior view of final segment of puparium, showing posterior larval spiracles, plsp, and anus.* FIG. 28. *Transverse section through the fore part of the abdomen of a parasitized fifth-stage Peronea larva, showing the Actia maggot within the sleeve. The microtrichia of the host cuticula have been omitted (see* FIG. 3*), as well as many of the Actia organs; dt, dorsal tracheal trunks of the maggot; sg, salivary gland of the maggot; mi, four cuts through the convoluted mid-intestine of the maggot.* FIG. 29. *Transverse section farther back than that shown in Fig. 28; cut through the head of the maggot (note the oral hooks). The pharynx and other organs of the maggot have been omitted in the drawing.*


wing
haltere
20
prothoracic cornicle
isp
haltere
21
pupal wall
spc
tt
23
22
a.l sp
A1
A8
25
larval mouth
24
am el
anus
sleeve
maggot
blood
sg
dt
ml
salivary ducts
28
26
p l sp
27
anus
transverse muscle
muscles
peritrophic mem
epithelial cells
salivary gland
blood
fat body
maggot
longitudinal muscle
29
sleeve

the first stage was outside the funnel, lodged against the body wall or alimentary tract of the host larva, from the prothorax as far back as the ninth abdominal segment.

From the above it is apparent that the integumental funnel is developed secondarily, not at the point of entrance of the newly hatched burrowing maggot, but rather as a response to irritation from within, which most often occurs during the second stage of the parasite, and the fifth stage of the host. The large hooked spinules on the posterior segment of the first- and second-stage *Actia* maggots are probably employed in rupturing the body wall, and in anchoring the maggot in the developing funnel. In one instance the funnel appeared in a host larva isolated for some days, and eight days later the full-grown maggot issued from the dead host.

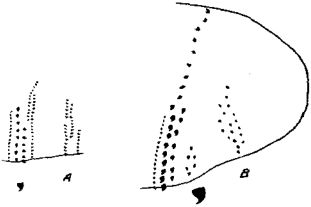


FIG. 30. *Spinules of the first-stage maggot. A. Those of the eighth transverse band, between the fourth and fifth abdominal segments. B. Those of the final abdominal segment.*

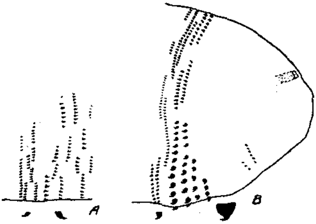


FIG. 31. *Spinules of the second-stage maggot. A. Those of the eighth transverse band, between the fourth and fifth abdominal segments. B. Those of the final abdominal segment.*

Of approximately one hundred *Peronea* larvae parasitized by *Actia*, which were dissected, only one contained more than one parasite maggot. This host larva had two small maggots, both of which were free in the body cavity.

The Integumental Funnel

Over a hundred *Peronea* larvae with the funnel have been examined, and in every instance the funnel was located on one side of the mesothorax (Fig. 1). The point of attachment was always within a restricted area, between the seta *theta* and the *kappa* group, either slightly above or below the horizontal line between these setae, or in some cases right on the pinaculum which bore the setae. The black disc is a thickened area in the host integument (Figs. 2 and 3), consisting of cuticula and hypodermis; these layers are readily distinguished in the basal part of the funnel. The ingrowing funnel bends abruptly and continues backward as a fine sleeve to one side of the alimentary tract. The hypodermal cells were not distinguished in the thin sleeve, under the oil immersion lens. The maggot rests within the sleeve, with the posterior segment curved into the basal funnel and the

posterior spiracles against the aperture. Whether the sleeve is closed at the free end is uncertain, but at any rate it has been observed in microtome sections beyond the tip of the maggot.

The early stages of *Actia* do little damage to the host, and even in the first part of the third instar, the chief abnormality seems to be crowding of the host intestine by the growing parasite (Fig. 28). During the last two or three days within the host, the maggot feeds at a destructive rate, and consumes nearly all the organs and tissues, except the tracheae and occasionally the alimentary tract. A day or more after the host has been killed, the maggot issues from the remains (through the anus in some cases that were observed), leaving the integumental funnel attached to the mesothorax.

A perplexing feature of the host-parasite relationship is the apparently invariable selection of the side of the mesothorax for the location of the secondary funnel. It may be that the young free-living maggot, when moved to establish a respiratory connection with the exterior, is governed by the absence of spiracles and the tracheal trunks leading from them, which would limit selection to the mesothorax, metathorax, and the last two abdominal segments. Of these four, the mesothorax would be most suitable as a point of attachment, as ample room for growth would be assured to the parasite, which would lie with its mouth near the source of rich nutriment, that is, near the region of the mid-intestine of the host. This, however, is only speculation.

Acknowledgments

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INFLUENCE OF WEATHER CONDITIONS ON THE NITROGEN CONTENT OF WHEAT[1]

By J. W. Hopkins[2]

Abstract

A statistical study of results from experimental plots of Marquis wheat grown annually (though not on the same soil) at five points in Saskatchewan and Alberta indicates that there was a significant negative correlation between the amount of rainfall during the growing season and the nitrogen content of wheat. The main effect of rainfall was exerted during May and June. The data do not justify the conclusion that the amount of rain falling in July or August, or the amount of pre-seasonal precipitation, modified the nitrogen content significantly. Mean maximum temperature for July or August failed to show a significant correlation with nitrogen content, but may not be a satisfactory measure of the temperature conditions actually experienced by the crop.

It is suggested that the preponderating effect of early rainfall may be due to the fact that it stimulates tillering and vegetative development generally. The available nitrogen must thus be distributed amongst an increased number of culms, whilst at the same time the total leaf area devoted to the production of carbohydrates is augmented.

1. Introductory

The present surplus of agricultural commodities has caused increased attention to be directed to the quality of Canadian wheat as a factor affecting export sales, with the result that information respecting the quality of the annual production may be as important as the statistics of quantity.

A high protein content is the most important single concomitant of wheat strength. It is generally conceded that weather conditions exert an important influence on the composition of the grain, as well as on the yield but, as yet, the quantitative aspects of these relations have not been definitely established for Canadian crops.

2. Experimental Data

Observational results were acquired through the courtesy of Dr. F. T. Shutt, who inaugurated, in 1912, an extensive experiment on the influence of environment on wheat quality. Each year, parent seed of the variety Marquis O-15, produced during the previous season at Indian Head, Saskatchewan, was distributed to Dominion Experimental stations throughout the country, sown in small plots, and five-pound samples of the resulting grain harvests sent to Ottawa for analysis. An account of the results obtained during the period 1912–1932, when the experiment was terminated, has been given by Shutt and Hamilton (6), together with certain conclusions deduced from variations in the average protein content of the grain produced at different stations. The results at individual stations in different seasons may, however, be utilized to study more directly the influence of weather conditions.

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From 1915 onwards, extensive notes on the previous history of the experimental plot, growth of the crop, incidence of frost, hail and disease, etc., as well as the total yield of grain secured, were made in a uniform manner at all stations. These proved to be of great value in the discovery of certain sources of variation and the elimination of results secured under obviously abnormal conditions.

TABLE I

YIELD OF GRAIN (LB. PER ACRE) AND NITROGEN CONTENT (%) OF WHEAT

Station	Year	Yield	N content	Station	Year	Yield	N content
Lacombe, Alberta	1915	3520	2.59	Indian Head, Saskatchewan	1926	3020	2.35
	17	3000	2.49		28	2650	2.38
	21	3260	2.87		29	1950	2.86
	22	1200	3.24		31	1180	3.13
	25	3450	2.70		32	1250	3.02
	28	2250	2.59				
	30	3720	2.99	Scott, Saskatchewan	1915	2880	2.82
	31	3720	2.47		17	1040	3.12
	32	4340	3.09		18	280	3.55
					21	1700	3.22
Lethbridge, Alberta	1915	3840	2.69		23	1800	2.84
	16	2910	2.71		24	590	3.01
	17	1500	2.95		25	1690	2.87
	21	540	3.05		26	1220	3.22
	22	1500	2.92		29	1040	3.47
	25	1540	3.47		30	2560	2.80
	26	2290	3.03		31	1630	2.98
	27	1270	2.39		32	1310	3.00
	28	2720	2.75				
	29	1500	3.64	Rosthern, Saskatchewan	1915	2770	2.91
	30	760	2.92		21	1760	3.23
	31	760	2.92		22	2600	2.96
	32	2140	2.98		24	720	2.94
					25	1880	2.66
Indian Head, Saskatchewan	1915	3880	2.80		26	820	2.93
	18	2200	2.93		27	2050	2.55
	24	1540	2.73		28	1980	2.91
	25	2820	2.58		29	900	3.24
					30	1840	2.68
					32	2080	3.13

Considering only stations, in the prairie provinces, examination of the above records revealed that the great majority of the experimental crops were grown on land which had been summerfallowed or had supported a "summerfallow substitute" crop, such as potatoes, in the previous season. At the two Manitoba stations, Brandon and Morden, however, a diversity of previous treatment occurred, including as alternatives to the elsewhere usual summerfallow the growing of crops such as wheat, oats, barley, alfalfa, sweet clover, corn, melons and white beans (manured). Since to these actual or potential sources of variation in the yield and composition of the succeeding crop must be added several severe infestations of black stem rust, it was judged that attention should be confined to the results obtained at stations in central and southern Saskatchewan and Alberta. These also were freed

of a number of crops grown in succession to legumes, of a further number affected by catastrophic occurrences, such as frost, hail or heavy disease infestation, and of still others of doubtful status owing to the absence or loss of the field records. The grain yields and nitrogen contents of the remainder, which are believed to be reasonably comparable, are shown in Table I. The meteorological data, with which these are to be correlated, were obtained from the Monthly Record of the Meteorological Service of Canada, supplemented by correspondence with the Director of that Service, and are given in Tables II, IV and V.

TABLE II

SUMMER PRECIPITATION (INCHES OF RAIN) BY MONTHS

Station	Year	May	June	July	Aug.	Station	Year	May	June	July	Aug.
Lacombe,	1915	1.25	8.28	2.79	0.84	Indian Head,	1928	0.64	6.05	2.14	0.34
Alberta	17	3.26	1.49	1.13	1.88	Saskatchewan	29	2.16	1.12	0.73	0.18
	21	1.69	1.85	3.28	0.98		31	0.23	1.18	1.87	2.38
	22	1.30	1.75	1.88	2.94		32	1.87	3.44	2.22	3.27
	25	1.53	1.51	1.32	3.87						
	28	0.50	7.30	1.66	2.56	Scott,	1915	1.40	3.54	2.11	0.48
	30	1.61	2.08	3.72	2.93	Saskatchewan	17	0.41	0.88	1.03	1.42
	31	0.86	8.11	2.59	2.53		18	0.19	0.29	1.87	0.93
	32	2.08	3.97	2.30	1.84		21	1.39	1.66	1.65	0.56
							23	0.95	5.67	4.25	1.45
Lethbridge,	15	3.03	4.84	3.44	0.96		24	1.10	0.58	0.70	2.87
Alberta	16	3.77	3.54	3.33	2.97		25	1.36	3.48	2.68	1.31
	17	0.95	1.42	1.37	2.00		26	2.97	1.39	0.75	2.52
	21	0.96	1.04	3.23	0.46		29	0.63	2.64	0.67	0.45
	22	0.89	1.87	2.30	0.40		30	0.38	2.79	2.45	1.08
	25	0.43	3.40	0.82	1.85		31	0.12	2.41	3.13	0.83
	26	0.64	4.67	1.15	2.31		32	1.82	2.83	2.78	1.30
	27	7.32	1.60	1.93	1.74						
	28	0.09	6.79	3.98	1.54	Rosthern,	1915	1.16	1.00	3.12	0.28
	29	2.63	3.72	0.52	0.59	Saskatchewan	21	1.88	1.49	4.91	0.64
	30	1.54	1.42	0.57	0.57		22	3.66	1.54	0.53	3.28
	31	1.22	1.55	1.09	0.19		24	0.70	0.87	0.77	3.73
	32	2.99	2.06	0.74	3.63		25	0.96	4.01	2.31	3.60
							26	3.38	0.48	0.79	1.48
Indian Head,	15	1.37	2.32	1.92	1.75		27	2.62	4.32	5.63	1.22
Saskatchewan	18	1.72	0.82	1.96	3.02		28	0.40	2.57	4.26	1.03
	24	0.36	2.22	1.05	3.00		29	1.13	3.05	0.72	0.81
	25	0.41	3.47	0.60	0.46		30	1.09	2.47	2.44	0.42
	26	3.07	1.99	1.25	1.97		32	0.71	3.68	3.16	2.43

3. Statistical Analysis

Seasonal Variance of Yield and Nitrogen Content

In order to eliminate any bias due to differences in average soil composition or weather conditions between stations, the quantities dealt with will be the seasonal deviations from the station averages, rather than the absolute values of yield and nitrogen content listed in Table I. Assuming the results obtained at different stations in the same year to be independent, the observations, 54 in all, provide 49 degrees of freedom for the estimation of the seasonal variance of yield and nitrogen content, and the covariance of these with rainfall, etc.

The yield of grain secured exhibits a seasonal standard deviation of 847 lb. per acre, corresponding to a coefficient of variation of 41.8%, the analogous values for nitrogen content being 0.86 and 29.6% respectively. An inspection of Table I will reveal that high yield is frequently associated with low nitrogen content and *vice versa*. There are however several exceptions to this tendency. The seasonal covariation of the two quantities yields a correlation coefficient of only −0.40, which, although significant, indicates that the fluctuations in the two attributes are far from strictly proportional.

Influence of Summer Rainfall

Table II shows the amounts of precipitation recorded at the various stations during the months of May, June, July and August of the seasons for which crop records are available in Table I. It is required to estimate from the data of Tables I and II the values of the regression coefficients b in the equation

$$(N-\bar{N}_s) = b_1(r_1-\bar{r}_{1s}) + b_2(r_2-\bar{r}_{2s}) + b_3(r_3-\bar{r}_{3s}) + b_4(r_4-\bar{r}_{4s})$$

where $(N-\bar{N}_s)$ represents the amount by which the nitrogen content in any given season is in excess or defect of the appropriate station average, and $(r-\bar{r}_{1s})$, $(r_2-\bar{r}_{2s})$, etc., represent the corresponding seasonal fluctuations of May, June, etc., rainfall. The normal equations to determine the coefficients b by the method of Least Squares are:—

$$\begin{aligned}
78.5671b_1 - 27.3152b_2 - 6.1063b_3 + 9.6777b_4 &= -4.5431\\
-27.3152b_1 + 169.8572b_2 + 40.0188b_3 - 11.3695b_4 &= -8.7296\\
-6.1063b_1 + 40.0188b_2 + 73.8206b_3 - 13.6903b_4 &= -3.6183\\
9.6777b_1 - 11.3695b_2 - 13.6903b_3 + 56.7984b_4 &= +.3672
\end{aligned}$$

giving

$b_1 = -.0804\%$ nitrogen per additional inch of May rainfall
$b_2 = -.0586\%$ nitrogen per additional inch of June rainfall
$b_3 = -.0234\%$ nitrogen per additional inch of July rainfall
$b_4 = +.0028\%$ nitrogen per additional inch of August rainfall

as the average effect of one additional inch of rain during the months specified.

Replacing the quantities on the right hand side of the above equations by the covariance of yield and rainfall, 6781.94, 31178.18, 18822.93 and −3640.17 respectively, enables the regression of grain yield on rainfall by months to be computed as follows:—

$b_1 =$ 160 lb. per acre per additional inch of May rainfall
$b_2 =$ 167 lb. per acre per additional inch of June rainfall
$b_3 =$ 175 lb. per acre per additional inch of July rainfall
$b_4 =$ −16 lb. per acre per additional inch of August rainfall.

The sum of the squares of the seasonal deviations of nitrogen content and yield, corresponding to 49 degrees of freedom, may now be partitioned as indicated in Table III.

TABLE III

Variance	Degrees of freedom	Nitrogen content		Yield	
		Sum of squares	Mean square	Sum of squares	Mean square
Accounted for by regression	4	.9625	.2406	9639000	2410000
Deviations from regression	45	2.6810	.05958	25478000	566200
Total	49	3.6435		35117000	

In both cases the regression function has accounted for a greater proportion of the total variance than is consistent with the hypothesis of non-association, indicating that a real correlation does exist. But it will be noted that whereas the yield of grain secured is influenced by the amount of rain falling in May, June and July, the major, and indeed the only definitely significant, effect upon nitrogen content appears to be exerted during the first two months of the growing season. The degree of association is only moderate, being measured by multiple correlation coefficients of $R=0.52$ and $R=0.51$ in the case of grain yield and nitrogen content respectively. This is not however altogether surprising in view of the fact that the crop was grown on different plots at each station each year.

As there is some degree of correlation (negative) between the precipitation recorded in May and June of the same year during the period under review, it is to be expected that a good approximation to the regression of nitrogen content on summer rainfall might be obtained by the use of the total precipitation for May and June only. The resulting regression coefficient has in fact the value -0.068% nitrogen for each additional inch of rain, and accounts for only a slightly smaller amount (0.9090) of the total sum of the squares of annual deviations in nitrogen content than the regression equation in which all four months are treated separately (0.9625).

Influence of July and August Temperature

It is suggested by Shutt and Hamilton (6) that scanty precipitation and high temperatures during the later weeks of development and ripening of the kernel, by drying out the soil, constitute the chief environmental factor conducive to high protein content.

The average daily mean temperatures recorded for the months of July and August at any particular station show but slight seasonal variation, and such variation as does occur is wholly uncorrelated with nitrogen content.

It was felt, however, that the mean daily maximum for the month might be a better measure of the temperature conditions actually experienced by the crop. The values of this quantity are listed in Table IV.

TABLE IV

MEAN MONTHLY MAXIMUM TEMPERATURE (DEGREES F.)

Station	Year	July	August	Station	Year	July	August
Lacombe, Alberta	1915	68	79	Indian Head, Saskatchewan	1928	76	74
	17	77	72		29	83	83
	21	76	76		31	81	78
	22	77	78		32	78	77
	25	80	72				
	28	75	70	Scott, Saskatchewan	1915	72	82
	30	78	76		17	82	76
	31	75	73		18	80	76
	32	73	76		21	78	78
					23	77	70
Lethbridge, Alberta	1915	70	82		24	79	69
	16	75	73		25	76	74
	17	83	76		26	80	72
	21	79	78		29	79	79
	22	76	80		30	76	77
	25	79	76		31	76	74
	26	82	72		32	74	76
	27	74	73				
	28	74	72	Rosthern, Saskatchewan	1915	70	80
	29	80	83		21	78	76
	30	80	81		22	78	77
	31	78	79		24	79	70
	32	80	77		25	78	75
					26	82	74
Indian Head, Saskatchewan	1915	70	80		27	76	75
	18	78	73		28	78	72
	24	79	72		29	80	80
	25	78	79		30	79	84
	26	83	74		32	76	76

Considering the crude data only, there is some apparent covariation of temperature and nitrogen content, above-average temperatures tending to be associated with above-average nitrogen. When, however, due allowance is made, by the method of partial correlation, for the influence of May and June rainfall, the covariance of nitrogen content and temperature is much reduced. The effect of July temperature variations is then measured by a partial regression coefficient of 0.008 ± 0.012, which is smaller than its standard error and hence statistically insignificant. The August partial regression coefficient is $0.017 \pm 0.0094\%$ nitrogen per degree F. Here there is perhaps some indication of a real effect, but as this coefficient also does not significantly exceed its standard error, no definite conclusion is justified.

Influence of Pre-seasonal Precipitation

In view of the fact that the maximum influence of summer rainfall on nitrogen content appears to be exerted in the first two months of the growing season, it might be thought that pre-seasonal precipitation would also have some indirect effect, through the initial supply of soil moisture thus made available.

The total precipitation, expressed as inches of rain, recorded from August 1 of the preceding year to April 30 of the crop year, is shown for the various stations and seasons in Table V. If a proper estimate of the regression of nitrogen content on pre-seasonal precipitation is to be obtained, allowance must be made for the effect of the associated May and June rainfall, which has already been shown to be significant. When this is done, the regression coefficient deduced is $-0.023 \pm 0.013\%$ nitrogen for each additional inch of precipitation in the period August 1 to April 30. This does not significantly exceed its standard error, so that although there is some indication of correlation, the reality of the effect cannot be said to be definitely established.

TABLE V

PRE-SEASONAL PRECIPITATION (INCHES OF RAIN), AUGUST 1 OF PRECEDING YEAR TO APRIL 30 OF CROP YEAR

Station	Crop year	Precipitation	Station	Crop year	Precipitation
Lacombe,	1915	7.64	Indian Head,	1928	12.99
Alberta	17	12.98	Saskatchewan	29	3.93
	21	6.64		31	4.53
	22	5.82		32	7.27
	25	11.72			
	28	12.22	Scott,	1915	11.44
	30	7.07	Saskatchewan	17	8.48
	31	7.27		18	5.28
	32	11.79		21	10.48
				23	7.04
Lethbridge,	1915	10.51		24	5.95
Alberta	16	7.99		25	11.86
	17	14.08		26	7.61
	21	5.73		29	3.83
	22	8.12		30	5.67
	25	13.06		31	5.99
	26	10.02		32	10.15
	27	11.87			
	28	13.43	Rosthern,	1915	7.08
	29	8.84	Saskatchewan	21	10.81
	30	10.11		22	7.24
	31	7.42		24	7.49
	32	9.92		25	11.41
				26	9.90
Indian Head,	1915	5.97		27	8.92
Saskatchewan	18	7.89		28	6.77
	24	7.78		29	6.05
	25	13.83		30	7.70
	26	8.85		32	9.73

It might perhaps be suggested that variations in winter snowfall have no great influence on soil moisture, and serve merely to mask any real correlation between nitrogen content and pre-seasonal rainfall. To test this point, calculations similar to the foregoing, but employing the precipitation during August, September, October, and the succeeding April only, were made. The resulting regression coefficient is practically unchanged at -0.026% nitrogen for each additional inch of rain, and still statistically insignificant.

4. Discussion of Results

The negative correlation between yield and nitrogen content of grain is not wholly novel, similar results having been reported, for example, by Waldron (8) and by Malloch and Newton (3), though in these cases the relation was deduced from the analysis of different strains or plots grown in the same season. There is, moreover, a general impression that in years of high yield the nitrogen content tends to be low, and *vice versa*. Average seasonal differences of this nature must be largely the result of weather conditions. As pointed out by Finnell (1), when soil differences constitute an important factor, increased yield is not necessarily associated with a diminished nitrogen content.

The meteorological correlations of the preceding section, however, do not wholly agree with the conclusions (in part derived from the same data) of Shutt and Hamilton (6). These authors infer, from a comparison of the results secured over a period of years at two stations in eastern Canada (Charlottetown, P.E.I. and Kentville, N.S.) and two stations in western Canada (Scott, Sask. and Invermere, B.C.), that the "chief environmental factor conducive to high protein content" is "the drying out of the soil, due to scanty precipitation and high temperatures, during the later weeks of development and ripening of the kernel."

It may be noted that these conclusions refer to average climatic differences between widely separate regions, rather than to the usually less pronounced annual fluctuations in the weather conditions of one locality. Alsberg and Griffing (2) have emphasized this consideration, but as they themselves point out, climate and weather are "not from their very nature separable," and it seems reasonable to expect the direct effects of climatic differences to be simulated, on a smaller scale, by the vagaries of the weather. When considering the results of Shutt and Hamilton (6), however, the indirect effect of permanent climatic differences, in modifying soil conditions, should be borne in mind. It also seems desirable to point out (i) that the previous treatment of the experimental plots at Charlottetown and Kentville (including the growing of corn, oats, mangels, turnips, potatoes, clover hay, peas and hemp) was not identical with that at Invermere and Scott, and (ii) that in addition to the differences in the average rainfall and maximum temperatures for July and August between the eastern and western stations, referred to by them, the former also have a significantly higher average May and June rainfall.

On the other hand, the results deduced in Section 3 agree well with those reported by Russell and Bishop (5) as the outcome of a study of the influence of weather conditions on the nitrogen content of barley grown at Woburn, Beds., from 1885 to 1926. Here, also, the major influence of rainfall was found to be exerted in the first half of the growing season, and the effect of temperature at all times appeared to be slight. Russell and Bishop suggest that excessive spring rains may leach out nitrates, which would otherwise

remain to be utilized by the plant at a later stage of development. This may well be the explanation of their results, but it seems unlikely that such reasoning will apply with equal force under the semi-arid conditions of western Canada. Above-average rainfall may indeed even here carry the already available nitrates to somewhat lower levels and, by lowering the temperature of the soil, temporarily retard the rate of nitrification; but it seems likely that the main effect is produced in another way.

Other work by the author has shown a definite correlation between rainfall during the early part of the growing season and the subsequent yield of grain, under Canadian prairie conditions, and it is suggested that this is due to the stimulation of tillering and general vegetative development of the plant. Smith (7) in fact reports a correlation of $+0.92$ between rainfall and amount of tillering. As a result of this proliferation, the supply of available nitrogen must be distributed amongst an increased number of culms, and the total leaf area, devoted to the production of carbohydrates, will also be augmented, both circumstances tending towards a diminished proportion of nitrogen in the resulting grain. The insignificant effect of later rainfall may be due in part to the fact that (i) most of the plant nutrients derived from the soil are taken up before blossoming (2), and (ii) as pointed out by Russell and Bishop (5), the ratio of carbohydrates to nitrogen in the translocated material, is, even in an unfavorable season, so high that very large additional amounts of the former would have to be produced by the plant in order to affect appreciably the composition of the grain. Variation due to soil differences between the plots employed in different seasons may also have obscured the significance of real, though less pronounced, weather effects.

The apparently meagre effect of temperature during the later stages of development is perhaps surprising, for quite apart from any possible influence of desiccation on the proportionate translocation of carbohydrate and protein, higher temperatures might be expected to affect the ratio of nitrogenous to carbonaceous material in the developing grain by increasing the rate of respiration. It is of course possible that such respiratory effects did not attain the magnitude, relative to other sources of variation, necessary for detection. However, respiration is known to increase in an approximately exponential manner with temperature up to the lethal point, so that a difference of one degree has a greater effect at high than at low temperatures; the use of a linear average may therefore have failed to reveal the true effect of relatively short periods of very hot weather. From the point of view of the desiccation theory also, the inadequacy of temperature records, confined to daily maxima and minima, as an indication of the actual conditions, especially those resulting from hot dry winds, should not be overlooked. The systematic compilation of evaporation measurements at agricultural experiment stations would undoubtedly provide data of considerable value for use in future agricultural meteorological studies.

A high degree of correlation with the meteorological data was hardly to be expected of crop records secured from different small plots each season, in view of the effects upon both yield and composition of crops known to be occasioned by soil differences. Thus to cite only one instance, Newton and Malloch (4) report coefficients of variability ranging from 8.2 to 15.1 for the protein content of replicate plots of wheat varieties grown on the same field in the same season. As elsewhere suggested, however, such fluctuations will, to a considerable extent, cancel out when the annual results from any considerable area are considered, and a correspondingly closer relation between weather conditions and the average quality of the crop may be anticipated.

Acknowledgment

The author wishes to record his appreciation of the courtesy of Dr. F. T. Shutt, in making available for study the observations accumulated by him over a period of many years.

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EFFECT OF HEAT TREATMENT ON THE VISCOSITY OF GLUTEN DISPERSED IN ALKALI, ACID AND NEUTRAL SOLVENTS[1]

BY W. H. COOK[2] AND R. C. ROSE[3]

Abstract

The viscosity of gluten dispersions in sodium hydroxide and acetic acid always decreased during heat treatment, the magnitude of the decrease being much larger in the former than in the latter solvent. Dispersions in urea solution decreased to a fixed viscosity level at temperatures below 70° C., but at higher temperatures the initial viscosity decrease of dilute dispersions was followed by an increase, then again by a decrease. At 60° C., dispersions in sodium salicylate increased in viscosity throughout the period of heat treatment, but at 80° C. the viscosity increased to a maximum and then fell off. This qualitative difference in the viscosity changes at temperatures above and below 70° C. in the neutral solvents, was the only evidence obtained to indicate that dispersed gluten undergoes any sudden change in character at a certain temperature, comparable to the so-called "coagulation point" of albumins. The results indicate that the changes occurring below 70° C. can be attributed mainly to the action of the solvent on the protein.

1. Introduction

There is considerable evidence to show that wheat and flour are affected by heat, certain treatments apparently improving the baking strength of the flour while others cause deterioration. Such treatments may affect several of the flour constituents, but it seems probable that the gluten proteins suffer the greatest change. Geddes (3, 4) has shown that the alteration in the proteins, due to heating, is evidenced by a decreased viscosity of leached, acidulated, flour-in-water suspensions, a decreased rate and extent of imbibition of the washed gluten, and a decrease in the ease of dispersion in neutral salt solutions. Herd (5) has also shown that the solubility of the proteins is decreased by severe heating, but the results of viscosity measurements on flour-in-water suspensions at pH 4.0–4.5 led him to the conclusion that severe heat treatment decreases the rate of swelling, but enhances the swelling power of the gluten.

The above evidence shows quite definitely that gluten protein in the solid state is rendered less soluble by heat treatment. This change has frequently been referred to as denaturation, but the exact nature of this phenomenon, even in such proteins as albumins which have been studied most extensively, is still obscure. It has been reported (1, 3, 4) that the extent of the change produced in gluten increases gradually with severity of heat treatment, although some of Geddes' (4) results suggest that over a certain range the

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reaction has a high temperature coefficient similar to the known heat denaturation reaction of albumins. Results obtained with gluten and albumin, however, are not strictly comparable, since, in the investigation cited, the gluten protein was in a solid form, whereas albumin is generally studied in solution. It has also been shown (3, 4, 5) that the moisture content of the flour has a decided effect on the extent of the change which occurs on heating, and the quantitative relations may be complicated further by the presence of different electrolytes and of starch.

The lower solubility of the gluten protein after heat treatment has sometimes been attributed to coagulation, although the loss of solubility could be equally well explained by such a change as dehydration. It is, however, practically impossible to determine from experiments performed on flour or washed gluten whether or not coagulation does occur. On the other hand, gluten dispersed in the classical solvents, dilute acid and alkali, shows no evidence of visible coagulation on heating, although it is highly probable that these reagents prevent coagulation, or tend to redisperse any coagulum that might form as the result of heating. Cook (2) found that a 3% dispersion of gluten in 30% urea solution decreased in viscosity during heat treatment at 70° C., a behavior which suggests that heating favors either dehydration or dispersion rather than coagulation. Here again it is probable that the observed change was due to the effect of the solvent on the protein, rather than to the effect of heat alone.

The object of this investigation was to study the effect of heat on the viscosity of dispersed gluten with a view of determining whether there was any evidence of protein coagulation in any of the solvents. The authors (9) have already shown that the viscosity changes of gluten dispersions are determined, to a large extent, by the solvent employed, and as it doubtless affects the changes induced by heat treatment, the four solvents, dilute sodium hydroxide, dilute acetic acid, urea and sodium salicylate, were again used.

2. Methods

The flour, from which the gluten was obtained, and the method of preparing, analyzing and determining the viscosity of the dispersions were the same as those recently described (9). The dispersions were heat treated in stoppered tubes as described by Cook (2), the period of treatment being measured from the time the tubes were put in the bath until the sample was removed and placed in a water bath at 25° C. In some of the later experiments an improved method was employed in which portions of the dispersion were heat treated in small sealed tubes which were not opened until they had been cooled to 25° C. In this way the small amount of evaporation, which necessarily occurred when the larger tubes were opened for sampling, was avoided.

Urea solutions tend to decompose and during heat treatment they become alkaline in spite of added buffer substances. This necessitated frequent determinations of the hydrogen ion concentration to insure that the viscosity

measurements were obtained within the pH-stability range (9). As the viscosity is also affected by the urea concentration, an experiment was made to determine to what extent its decomposition might affect the results. It was found that heating a 30% urea solution for four hours at 80° C. resulted in an evolution of ammonia equivalent to about 0.5% of the urea nitrogen present. This would reduce the urea concentration only from 30 to 29.85% and it is evident (9, Fig. 2) that such a change would not affect the viscosity significantly.

The viscosity behavior of gluten dispersions during storage at 0 and 25° C. has been reported (9), and all the dispersions used in the present study were allowed to attain a reasonably constant viscosity before being heated. Initially, it was thought best to store the dispersions at 0° C. and, as urea solutions were employed first, all the dilute dispersions in this solvent, with the exception of the one that was treated when 30 days old, were stored at this temperature prior to heat treatment. All others were stored at 25° C. Dispersions in urea were heat treated at 40, 60, 70, 80, 90 and 100° C. but, in view of the results obtained, dispersions in the other solvents were treated at 60 and 80° C. only.

3. Results

Changes in Viscosity

The viscosity changes with time and temperature of heat treatment of gluten dispersions in all four solvents are shown in Figs. 1, 2 and 3. The concentration of the dispersions in mg. of protein nitrogen per gram of dispersion and the temperature of treatment are indicated in each figure. The number *at the end of each curve gives the age of the dispersion in days, at the time heat treatment was started.*

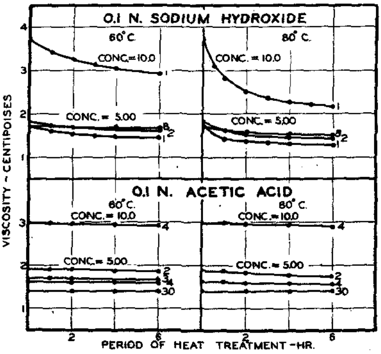

FIG. 1. *Effect of heat treatment on the viscosity of dispersions in dilute alkali and acid.*

The curves in Fig. 1 show that in all cases the viscosity of dispersions in sodium hydroxide decreased with time of heat treatment, the decrease being greater at 80° C. than at 60° C., and greater for concentrated than for

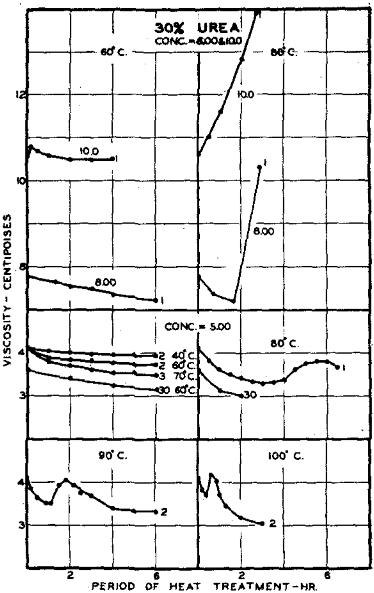

FIG. 2. *Effect of heat treatment on the viscosity of dispersions in urea solution.*

dilute dispersions. Of the latter, that which was eight days old when heat treated changed less than those which were only one or two days old. The viscosity of dispersions in acetic acid decreased slightly but showed much less change than in sodium hydroxide. The effect of heat treatment was practically the same in concentrated and dilute dispersions, and in dispersions of different ages.

It is evident from Fig. 2 that the viscosity of dispersions of 5.00 and 8.00 mg. of protein nitrogen per gram in urea solution decreased during heat treatment at temperatures of 70° C. or lower. At higher temperatures, and at 60° C. with the most concentrated dispersion, an increase in viscosity was observed at some stage of the heat treatment. The dilute dispersion that had been stored for 30 days at 25° C., prior to heat treatment, behaved in essentially the same way as the one that had been stored for two days at 0° C.

The results plotted in Fig. 3 show that the viscosity of dispersions in sodium salicylate increased throughout the entire period of heat treatment at 60° C., but at 80° C. it increased to a maximum and then decreased. At both temperatures the changes were greater in the concentrated dispersions. Here again the dispersion that had been stored for 30 days behaved in a similar manner to those that had been stored for two days.

Changes in pH

During the heat treatment, the changes in the hydrogen ion concentration of all dispersions were followed and the initial and final values are given in Table I. The duration of treatment was usually six hours, but with certain

dispersions in urea solution it was shorter, as shown by the curves in Fig. 2. The pH of dispersions in sodium hydroxide decreased during heat treatment, the decrease being greater at the higher temperature and in the more concentrated dispersion. In dispersions in acetic acid and sodium salicylate no significant change in pH occurred, whereas in urea solution the pH increased, a result which can be attributed entirely to the decomposition of urea. As it has already been shown (9) that the viscosity is affected by the pH when it exceeds 9.2, no viscosities of dispersions in urea solution have been reported where the pH exceeded this value.

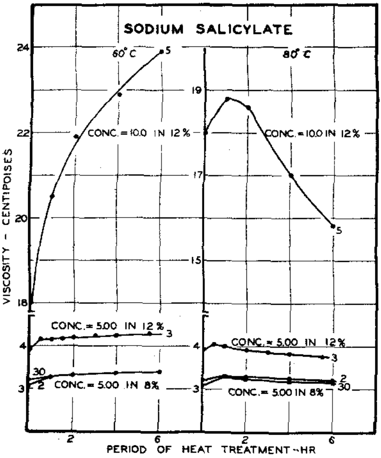

FIG. 3. *Effect of heat treatment on the viscosity of dispersions in sodium salicylate solution.*

TABLE I

CHANGES IN HYDROGEN ION CONCENTRATION DURING HEAT TREATMENT

Solvent	Concentration, mg. of protein N per gm.	Age of dispersion when heat treated, days	Initial pH	pH after treatment at 60° C.	pH after treatment at 80° C.
0.1 *N* Sodium hydroxide	5.00	1	12.57	12.30	11.84
	5.00	2	12.50	12.30	11.81
	5.00	8	12.00	11.92	11.20
	10.00	1	12.24	11.51	10.50
0.1 *N* Acetic acid	5.00	2	3.75	3.70	3.73
	5.00	3	3.72	3.54	—
	5.00	4	3.74	3.77	3.81
	5.00	30	3.77	3.76	3.75
	10.00	4	4.02	4.06	4.05
30% Buffered urea	5.00	2	6.82	7.21	8.74*
	5.00	30	7.55	7.73	8.05
	8.00	1	6.87	7.16	7.88
	10.00	1	6.79	6.95	7.72
8% Sodium salicylate	5.00	2	6.79	6.76	—
12% Sodium salicylate	5.00	3	6.90	6.86	6.89
	10.00	5	6.82	6.82	6.83

*Dispersion one day old.

Salting-out Tests

A number of salting-out tests were made on dilute dispersions in the neutral solvents after various heat treatments. The results are given in Table II. Heat treatment of dispersions in urea solution at 40° C. had little effect on the amount salted out, but diminished it if anything, whereas heating at 70 and 80° C. increased the amount precipitated. Heating at 60° C. had no effect on dispersions in 8% sodium salicylate but after heat treatment at 80° C. no precipitate was obtained.

TABLE II

PROTEIN NITROGEN SALTED-OUT BY MAGNESIUM SULPHATE FROM DILUTE DISPERSIONS AFTER VARIOUS HEAT TREATMENTS

Solvent	Heat treatment	Viscosity, centi-poises	$MgSO_4$ added to 10.0 ml.	Protein nitrogen precipitated	
				mg.	%
30% Urea	Nil	3.99	2.75 ml. of 20%	14.0, 16.8	28.0
	9.5 hr. at 40° C.	3.91	2.75 ml. of 20%	13.8, 11.2	22.7
	2 hr. at 70° C.	3.58	2.75 ml. of 20%	23.5, 23.7	42.9
	1 hr. at 80° C.	3.51	2.75 ml. of 20%	26.2, 26.1	47.5
8% Sodium salicylate	Nil	3.09	1.50 ml. of 5%	12.5, 12.2	22.6
	6 hr. at 60° C.	3.41	1.50 ml. of 5%	12.9, —	23.5
	6 hr. at 80° C.	3.21	1.50 ml. of 5%	0.0, 0.0	0.0

Stability of Viscosity Following Heat Treatment

A number of dispersions in urea solution were stored at 0° C. after being heat treated, and the viscosity determined periodically. The results, given in Table III, show that the viscosity remained constant during storage. This study did not include any dispersions that had increased in viscosity during heat treatment, so no information was obtained as to the stability of these systems.

TABLE III

VISCOSITY OF DILUTE DISPERSIONS IN 30% UREA SOLUTIONS STORED AT 0° C. AFTER HEAT TREATMENT

Heat treatment	Duration of storage at 0° C., days	Viscosity, centipoises	pH	Heat treatment	Duration of storage at 0° C., days	Viscosity, centipoises	pH
9.5 hr. at 40° C.	0	3.91	6.88	8 hr. at 70° C.	0	3.17	7.99
	1	3.92			0.2	3.13	
	3	3.94			1	3.10	
	10	3.90	6.87		2	3.11	
2 hr. at 70° C.	0	3.58	7.11		8	3.13	7.89
	1	3.55		1 hr. at 80° C.	0	3.51	7.20
	3	3.56			1	3.49	
	10	3.55	7.10		3	3.49	
4 hr. at 70° C.	0	3.38	7.44		10	3.50	7.35
	0.4	3.37					
	1	3.39					
	2	3.39					
	8	3.39	7.49				

Order of the Reaction

An attempt was made to determine the order of the reaction causing the viscosity decrease by calculating the time required for one-half of the viscosity decrease in dispersions of different concentrations. Since dispersions could be prepared and centrifuged at 25° C. only, the data obtained from experiments conducted at this temperature were employed (9, Fig. 5). Even at this temperature, the viscosity changes in sodium hydroxide and sodium salicylate were too small to permit a reliable estimation of the order of the reaction in these solvents. The results obtained with dispersions in acetic acid and urea, given in Table IV, show that the time required for half the viscosity fall-back was *independent of the initial concentration. The reaction is therefore* unimolecular.

TABLE IV

ORDER OF REACTION CAUSING VISCOSITY DECREASE

Solvent	Concentration, mg. of protein N per gm.	Initial viscosity, centipoises	Final viscosity,* centipoises	Time required for one-half of viscosity decrease, hr.	Reaction
0.1 N Acetic acid	5.10	3.68	1.56	10.0	Unimolecular
	5.46	4.13	1.62	10.0	
	10.3	8.70	2.90	10.0	
30% Urea	5.24	4.70	3.84	4.0	Unimolecular
	9.48	12.4	11.0	3.5	
	10.7	15.0	12.4	4.0	

**Final viscosity in acetic acid taken when dispersions six days old; final viscosity in first two dispersions in urea taken when they were two days old, and in last dispersion when 1.2 days old.*

4. Discussion

In the experiments of this type, the changes observed during heat treatment are due, not only to the action of heat, but also to the action of the solvent on the protein. It seems probable that, wherever the changes observed at the higher temperatures differed only quantitatively from those observed at 0 or 25° C., they were due to the action of the solvent. Qualitative differences, however, may reasonably be attributed to the action of heat.

Employing this criterion, the viscosity changes observed during the heat treatment of dispersions in alkali and acid can be attributed entirely to the action of the solvent. The pH decrease observed during the heat treatment of dispersions in sodium hydroxide indicates the exposure of more base-binding groups, and if Wu and Chen's (10) hypothesis is correct these results suggest that denaturation took place without coagulation.

In urea and sodium salicylate solutions the changes induced by heat treatment at temperatures below 70° C. can also be attributed to the action of the solvent, heat merely accelerating the reaction observed at lower temperatures.

The viscosity of dispersions in sodium salicylate increased more rapidly on heat treatment at 60° C. than during storage at 25° C. (9), and in concentrated dispersions the high viscosity attained at both temperatures suggests that some form of coagulation took place. The salting-out tests conducted on heat treated dispersions in this reagent are of little help in interpreting the changes in viscosity, but they lend support to the view that the reactions occurring at 60° C. and 80° C. are qualitatively different (Table II).

Dilute dispersions in urea solution were exposed to several temperatures below 70° C., and it is evident from the results (Fig. 2) that the final viscosity is apparently dependent on the temperature. A further study of this relation was made by computing the specific hydrodynamic volume of 1 mg. of protein nitrogen by means of Kunitz's (6) equation and plotting the values against the temperature of treatment. At temperatures of 40, 60 and 70° C., the final viscosity at the end of the heat treatment was used in the computation, and at 25° C. the value after storage for 20 hr. (9, Fig. 5) was used. As dispersion in urea could not be effected at 0° C., the probable specific hydrodynamic volume after dispersion and storage at this temperature was obtained by extrapolating from the constant values obtained during storage at 0° C., after initial periods of 2, 4, 6, 8, and 12 hr. at 25° C. (9, Fig. 4). The results are given in Fig. 4 along with the specific hydrodynamic volume when the viscosity was at a minimum, and at a maximum, at temperatures above 70° C. The fact that the values of the specific hydrodynamic volume at all temperatures up to and including 60° C. lie on a smooth curve is taken as evidence that the viscosity decrease observed at these temperatures is due to a continuation of the dispersing action of the solvent.

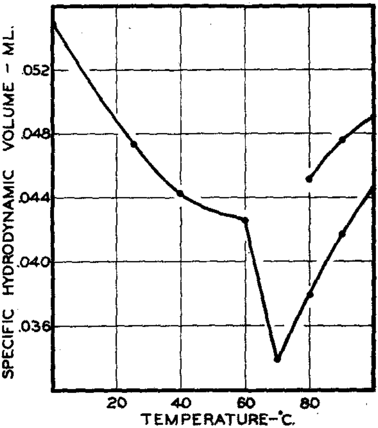

FIG. 4. *Effect of temperature on the specific hydrodynamic volume of 1 mg. of protein nitrogen in dilute dispersions in urea.*

This conclusion is supported by the fact that gluten dispersions were less easily salted-out after storage at 25° C. (9) and heat treatment at 40° C. (Table II). This behavior also indicates that the decrease in viscosity which occurred under these conditions was due to a further dispersion of the gluten rather than to dehydration, for, had the latter occurred the protein would have been more easily salted-out.

It has already been shown that the reaction causing the viscosity decrease at 25° C. in urea solutions is unimolecular, and doubtless the dispersion reaction at all temperatures below 70° C. is of the same order.

Even at temperatures above 70° C., where the effect of heat is most pronounced, it is evident from Figs. 2 and 3 that the solvent has a decided effect on the nature of the viscosity changes. Thus the viscosity of dispersions in sodium salicylate at first increased and then decreased, while that of dispersions in urea solution decreased, then increased to a maximum, and finally decreased again. The quantitative differences in the viscosity changes above and below 70° C. *are not as pronounced with dispersions in sodium salicylate* as in urea solution. The initial viscosity increase observed in the former solvent at 80° C. is interpreted as indicating a continuation of the same reaction which occurred at lower temperatures, while the final viscosity decrease suggests a further dispersion of the original material or a redispersion of the aggregates formed during the initial period.

Regarding the nature of the changes which occur in dilute dispersions in urea solution at temperatures of 70° C., or higher, it is evident that the specific hydrodynamic volume is at a minimum at 70° C. (Fig. 4), and from this it is concluded that dehydration takes place prior to the coagulation reaction, which causes the viscosity increase observed at higher temperatures. This conclusion is supported by the results of salting-out tests which show that the protein is more easily precipitated from dispersions heated for 1 hr. at 70° C. or 80° C. than from dispersions held at lower temperatures for the same period. As no increase in viscosity occurred under these conditions, the increased instability of the dispersions must be attributed to dehydration rather than coagulation.

The viscosity increase in dilute dispersions in urea solution which follows the initial decrease indicates that after dehydration reached a definite stage, coagulation set in. The viscosity decrease subsequent to the rise may be due to a redispersion of the coagulum and it seems likely that the several reactions proceed to some extent simultaneously. The period required for the viscosity to reach either a minimum or a maximum decreased as the temperature increased. Thus a maximum viscosity was obtained in about 5.5 hr. at 80° C., 1.8 hr. at 90° C. and 0.6 hr. at 100° C. As each additional 10° C. rise in temperature decreased the time required to reach a maximum to about one-third of its former value, the Q_{10} for these reactions is evidently about 3. The interval required for the dispersion to attain a maximum viscosity was used to calculate the critical increment of the process. This was found to be 177,000 cal. Lewis found the critical increment for the heat denaturation of haemoglobin to be 77,500 cal. (7) and of egg albumin 130,000 cal. (8). Considering the magnitude of these values and the relatively large difference between the values for albumin and haemoglobin, the above value for gluten is not at all improbable.

Considering all the results there appear to be four reactions which contribute to the viscosity changes, namely, dispersion, dehydration, coagulation and

redispersion, and it is possible that secondary changes such as hydrolysis also play a role. In alkali and acid there is no evidence of coagulation, and from the present results it is impossible to determine the number of reactions affecting the viscosity. In concentrated dispersions in sodium salicylate the viscosity increase is sufficiently marked to suggest coagulation, and this reaction may also cause the viscosity increase observed in dilute dispersions in this same reagent. At 80° C., coagulation is apparently followed by redispersion. In dilute dispersions in urea solution the dispersion reaction predominates at temperatures up to and including 60° C. At higher temperatures dehydration apparently sets in and it is followed by coagulation, the final reaction being a redispersion of the coagulum. With concentrated dispersions in this reagent coagulation may occur with no previous indication of dehydration.

The conclusion that gluten undergoes the greatest change at temperatures of 70° C. or higher is in agreement with the results of baking tests and viscosity measurements reported by Geddes (4). These show that flour heated for eight hours at 155° F. (68.3° C.) was not altered nearly as much as flour heated for a similar period at 160° F. (71.1° C.). It appears therefore, that gluten dispersed in the neutral solvents, particularly urea solution, is affected by heat in the same way as flour. Since this is not true of dispersions in alkali or acid, it is concluded that these solvents alter the protein to such an extent that it no longer exhibits the properties of native gluten.

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HYDROLYSIS OF GLUTEN INDUCED BY THE SOLVENT[1]

By W. H. Cook[2] and R. C. Rose[3]

Abstract

Dilute sodium hydroxide and dilute acetic acid both hydrolyze gluten even at 0° C., the reaction constant and temperature coefficient being greatest in dispersions in alkali. The reaction appears to be unimolecular in both reagents although it seems probable that neither of these can effect complete hydrolysis. No appreciable hydrolysis occurred in gluten dispersed in urea or sodium salicylate solutions, even after relatively drastic heat treatment.

1. Introduction

A study has been made of the relative extent to which dispersed gluten is hydrolyzed by different solvents during storage at various temperatures. Four dispersing agents were employed, namely, 0.1 *N* sodium hydroxide, 0.1 *N* acetic acid, 30% urea and 8% sodium salicylate, representing the solvents that are known to be capable of dispersing gluten completely. There is considerable evidence that dilute alkali and acid, in addition to causing denaturation, also induce secondary changes such as hydrolysis in proteins. Thus dilute alkali is known to cause decomposition of albumin (6), haemoglobin (8) and gluten (1); and acid, although its action may be less drastic, decomposes edestin (7) and other proteins (8). The neutral solvents may also denature proteins but experiments already reported (5) indicate that they alter the original properties of gluten less than dilute alkali and acid. The main object of this study was to determine which of the gluten solvents caused least hydrolysis, and also to see whether this secondary change was related to the alterations in viscosity already reported (2, 5).

2. Experimental

Methods

Dispersions containing 5 mg. of protein nitrogen per gram were prepared as described by Rose and Cook (5), subjected to various treatments, and an attempt was then made to determine, by precipitation methods, the proportion of the protein that had been hydrolyzed. Both tannic and trichloracetic acid were used as precipitating reagents for all dispersions excepting those in urea, where tannic acid only was used since trichloracetic acid had proved unsatisfactory (5). Hiller and Van Slyke (3) have shown that 2.5% trichloracetic acid is suitable for separating proteins from their split products, while Lundin and Schröderheim (4) have shown that tannic acid precipitates some peptones in addition to proteins.

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, and the Department of Field Crops, University of Alberta, Edmonton, Canada. Published as paper No. 67 of the Associate Committee on Grain Research.
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Dispersions in urea were analyzed as previously described (5), the precipitated nitrogen being determined directly. With the other solvents the nitrogen content of the filtrate was determined in the initial experiments, but later it was found that with aged dispersions in sodium hydroxide the odor of ammonia was evident, and here again it was considered more accurate to determine the nitrogen content of the precipitate. The weight delivered by a 20 ml. pipette was determined and this amount of dispersion added to 180 ml. of a solution containing sufficient precipitating reagent to make the final concentration 2.5% trichloracetic acid or 2.0% tannic acid. Precipitation with tannic acid left a clear supernatant liquid which was filtered directly into a Kjeldahl flask. As the supernatant liquid from the trichloracetic acid precipitations was cloudy, it was centrifuged and decanted, then brought to a boil and allowed to stand overnight at room temperature before filtering. This procedure yielded a clear filtrate and it is unlikely that it caused any significant hydrolysis, since Hiller and Van Slyke (3) found that boiling for 15 min. had no measurable effect.

The addition of the acidic precipitating reagents always caused a precipitation of salicylic acid from dispersions in sodium salicylate, and it was at first thought that this might interfere with the quantitative separation of the protein and non-protein nitrogen. In early attempts to overcome this difficulty, sufficient alcohol was added to keep the salicylic acid in solution, but it was found that the protein could not then be precipitated quantitatively from these solutions, and neither filtering nor centrifuging would render the supernatant liquid clear. An effort was then made to determine whether the precipitation of salicylic acid actually had any effect on the results. A dispersion in sodium hydroxide containing 5 mg. of nitrogen per gram was analyzed, and it was found that 74% of this nitrogen was precipitated by tannic acid. Ten grams of sodium salicylate was then added to 90 ml. and the dispersion analyzed as before. It was found that 76% of the nitrogen was precipitated. These results indicated that the precipitation of salicylic acid along with the protein had little effect, and no attempt was made to prevent its precipitation in subsequent experiments.

Results

The amount of nitrogen not precipitated by tannic acid and trichloracetic acid from gluten dispersions in sodium hydroxide, acetic acid and sodium salicylate, after various storage and heat treatments, is reported in Table I. The results obtained with dispersions in urea solution are given separately in Table II, owing to the different method by which these were obtained. These show definitely that under similar conditions, the amount of gluten hydrolyzed by the different dispersing agents decreases in the order, sodium hydroxide, acetic acid, and the neutral solvents, there being no significant hydrolysis by either of the last-named. In urea solution the results indicate that the original analysis was about 1% too low, while the slight progressive increase in the protein concentration following heat treatment can probably be attributed to evaporation or experimental error. In this experiment the

nitrogen content of the filtrate from dispersions in sodium hydroxide was determined, and as a loss of ammonia may have occurred these results are possibly somewhat low. Tannic acid always precipitated more nitrogen than trichloracetic acid, as would be expected, since the former reagent is known to precipitate such degradation products as peptones while the latter precipitates only proteins (3, 4). The difference between the amount of nitrogen precipitated by the two reagents suggests that the hydrolysis is a general disintegration of the protein molecule, rather than the splitting off of terminal amino acids or amide groups, for, had the latter occurred the amount of nitrogen precipitated by each of the reagents would have been approximately the same. The amount of nitrogen not precipitated by tannic acid is about 30% of that not precipitated by trichloracetic acid from dispersions in alkali and about 40% from dispersions in acid (Table I). This suggests that the degradation effected by dilute alkali differs from that caused by dilute acid.

TABLE I

HYDROLYSIS OF DISPERSED GLUTEN

Treatment	Solvent	Percentage of total nitrogen *not precipitated*	
		By tannic acid	By trichloracetic acid
Stored for 6 hr. at 25° C.	0.1 *N* Sodium hydroxide 0.1 *N* Acetic acid	1.9 1.4	7.0 3.4
Stored for 7 hr. at 25° C.	8% Sodium salicylate	0.8	—
Stored for 8 days at 25° C.	0.1 *N* Sodium hydroxide	12.9	30.9
Stored for 30 days at 25° C.	0.1 *N* Acetic acid 8% Sodium salicylate	9.0 1.0	31.0 1.1
Stored for 21 days at 0° C.*	0.1 *N* Sodium hydroxide	6.8	17.1
Stored for 36 days at 0° C.*	0.1 *N* Acetic acid	3.5	11.2
Treated at 60° C. for 6 hr. after 2 days at 25° C.	0.1 *N* Sodium hydroxide 0.1 *N* Acetic acid 8% Sodium salicylate	11.6 3.4 0.8	37.0 10.8 1.0
Treated at 80° C. for 6 hr. after 2 days at 25° C.	0.1 *N* Sodium hydroxide 0.1 *N* Acetic acid 8% Sodium salicylate	22.0 4.0 0.9	54.0 11.6 1.3
Treated at 60° C. for 6 hr. after 8 days at 25° C.	0.1 *N* Sodium hydroxide	16.2	41.8
Treated at 60° C. for 6 hr. after 30 days at 25° C.	0.1 *N* Acetic acid 8% Sodium salicylate	9.7 1.0	32.8 1.0
Treated at 80° C. for 6 hr. after 8 days at 25° C.	0.1 *N* Sodium hydroxide	21.2	51.9
Treated at 80° C. for 6 hr. after 30 days at 25° C.	0.1 *N* Acetic acid 8% Sodium salicylate	9.6 1.2	33.4 1.4

***An initial period of eight hours at 25°C. preceded the storage at 0° C.**

TABLE II

RECOVERY OF GLUTEN FROM DISPERSIONS IN 30% UREA SOLUTION

Treatment of dispersion	Percentage recovery
Stored for 30 days at 25° C.	101.0
Treated at 60° C. for 6 hr. after 2 days at 25° C.	101.0
Treated at 60° C. for 3 hr. after 30 days at 25° C.	101.3
Treated at 80° C. for 1 hr. after 30 days at 25° C.	100.7
Treated at 80° C. for 2 hr. after 30 days at 25° C.	101.5
Treated at 80° C. for 3 hr. after 30 days at 25° C.	102.0

Another experiment was performed in an attempt to determine the order of the hydrolysis reaction induced by dilute alkali and acid. Trichloracetic acid was the only precipitating reagent employed and the precipitate from dispersions in sodium hydroxide was analyzed directly in order to avoid the error due to the possible loss of ammonia. The results obtained after heating for various periods at 30 and 80° C. are given in Table III. These dispersions were only four hours old when the first determination was made, and it is evident that appreciable hydrolysis had occurred at this time.

TABLE III

RATES OF HYDROLYSIS

Solvent	Temperature of treatment, °C.	Duration of treatment, hr.	Concentration, percentage recovery	$K \times 10^6$ (unimolecular)
0.1 *N* Sodium hydroxide	30	0	88.0	
		50	72.4	
		122	62.4	1.7
0.1 *N* Sodium hydroxide	80	0	88.0	
		1.1	53.6	
		2	46.4	
		3	38.8	
		6	39.5	
		9	27.8	
		18	26.8	150
0.1 *N* Acetic acid	30	0	93.0	
		50	86.3	
		122	75.2	
		199	71.0	
		336	64.4	0.55
0.1 *N* Acetic acid	80	0	93.0	
		9	88.7	
		18	87.6	
		27	85.4	
		50	81.0	1.2

Neither the logarithm nor the reciprocal of the protein concentration gave a straight line when plotted against time, from which it appeared that the reaction was neither unimolecular nor bimolecular. It can be seen from the table, however, that at 80° C. the hydrolysis reaction in sodium hydroxide was apparently complete in nine hours, at which time about 27% of the original

nitrogen was still in the form of protein. It appears therefore that dilute sodium hydroxide is not capable of carrying the reaction to completion. In consequence the logarithm of the quantity (C-27), where C is the concentration given in Table III, was plotted against time. This gave a linear relation between the two quantities at both 30 and 80° C., indicating that the reaction was unimolecular. Unfortunately, the experiment with dispersions in acetic acid was not extended over sufficient time to obtain the limiting value, but when the logarithm of the quantity (C-27), as defined above, was plotted against time, the relation was practically linear. Since the reactions are apparently unimolecular, the appropriate reaction constants were calculated and are reported in Table III. From these it is evident that the hydrolysis of dispersions in sodium hydroxide is more rapid, and is accelerated more by a rise in temperature than that of dispersions in acetic acid. The results given in Table I confirm this conclusion.

3. Discussion

These findings are, to some extent, in agreement with the results of viscosity measurements (2), the decrease in viscosity during heat treatment being much greater in sodium hydroxide than in acetic acid. The extent of hydrolysis does not parallel the decrease in viscosity in all cases, however, as the viscosity of dispersions in sodium hydroxide did not change significantly during storage at 25° C., although quite extensive hydrolysis occurs under these conditions. Dispersions in acetic acid showed the reverse behavior, *i.e.*, a decrease in viscosity but comparatively little hydrolysis. If the latter is the only factor affecting the dispersions after several days storage, the viscosity behavior might be explained by assuming that the complexity of the resulting degradation products differed in the two solvents.

Since the present study indicates that hydrolysis does not occur in the neutral solvents, it is doubtful whether the viscosity changes previously observed (2, 5) can be attributed to this effect. Those viscosity decreases must, therefore, have been due to dispersion or dehydration.

It has already been shown (2, 5) that gluten dispersed in the neutral solvents retains the properties of the native protein to a higher degree than do dispersions in dilute alkali or acid. Whether this change brought about by the latter solvents is due to a primary (dispersion and denaturation) or to a secondary (hydrolysis) alteration of the protein is not known, but it is evident from the present results that drastic secondary changes occur in both alkali and acid in a remarkably short time.

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THE TREATMENT OF HYDRATED LIME WITH ALUMINIUM SULPHATE[1]

By A. F. Gill[2] and T. H. Way[3]

Abstract

This paper deals with the question of "workability" of hydrated limes for finishing purposes. The authors subscribe to the "colloid" conception of the mechanism underlying the well known superiority of undried putties over dry hydrates, on the one hand, and of magnesian or dolomitic hydrates over those high in calcium, on the other.

A description is given of an investigation made to determine whether an artificial gel could be applied to particles otherwise of poor quality by allowing them to react with aluminium sulphate. It was anticipated that the calcium hydroxide would react to form calcium sulphate and give a precipitate of aluminium hydroxide which, being relatively insoluble in the alkaline medium, would tend to coat the particles of calcium sulphate and residual calcium hydroxide. The effect of additions under different conditions was observed quantitatively by means of a flow-table plasticimeter. Favorable laboratory results were followed by practical trials which successfully demonstrated the usefulness of this treatment.

The value of hydrated lime for use as finishing plaster depends in large measure on that property, variously called "plasticity," "workability" or "fatness," which allows it to spread readily under the trowel, without "tearing" or "rolling", to give a smooth uniform surface. In this respect magnesian or dolomitic hydrates are usually superior to those high in calcium although the latter, other things being equal, are superior when slaked with an excess of water and used without being dried. The possibility of improving the properties of hydrated high-calcium lime to render it as valuable as dolomitic material for plaster finishing has long been of interest to owners of small lime plants, and indeed to those of many larger ones to whom dolomitic stone is not readily available.

The authors subscribe to the view that workability of a lime depends upon the presence in it of a major proportion of particles of colloidal size surrounded by a lubricating layer of adsorbed water (7). Such a conception serves to explain various physical and mechanical phenomena characteristic of desirable limes, such as rate of settlement from water suspension, "volume in water," and the important factor of water retention when applied to porous backings.

The fact that a hydrate, once dried, cannot be greatly improved by aging in water points to the view that the particles function as an irreversible colloid. That being the case, it is not surprising that no radical improvement in the quality of dry hydrates is obtainable through changes in methods of preparation.

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Attempts have been made by various investigators to improve hydrated lime through the addition of chemicals to the limestone (3), to the quicklime (4) or to the dry hydrate (2). Though certain improvements have been reported, no treatment of this kind, as far as the authors are aware, is being used commercially.

Consideration of this matter led to the opinion that the best possibility lay in an addition to the hydrated lime, subsequent to its preparation, of a reagent which would affect its properties in a mechanical way. It was recognized that additions of such materials as diatomite, bentonite, etc., had not yielded any great deal of success. Some success was attained in this laboratory by the use of crysotile asbestos fibre of "floats" grade. This material is very absorptive of water and, what is probably more important, has a rough fibre surface which would tend to hold lime particles apart. The chief disadvantage in its use lies in the fact that it is generally of a slate gray color and would therefore seriously detract from the salability of any hydrate with which it was mixed.

It was considered that an ideal admixture would be a material which would precipitate an artificial "gel" around the lime particles. This suggested the possibility of using aluminium sulphate. It was recognized that the calcium hydroxide would react with this material to precipitate calcium sulphate and gelatinous aluminium hydroxide. The latter appeared to have the specific property desired, while the calcium sulphate, being chemically identical with the gypsum commonly used with hydrated lime as gauging plaster, would do no harm. In addition, the products of this reaction are white, and thus would have no deleterious effect on the color of the product.

Qualitative trials made in the laboratory indicated that the proposal was technically feasible. It appeared, however, that an amount approaching 10% of the weight of a high-calcium hydrate would be necessary in many cases to give sufficient improvement. For this reason, and because information had been obtained that aluminium sulphate had been used by other investigators, generally with the object of increasing the strengths (1, 5, 6, 8), the matter was dropped for some time. Owing, however, to the receipt of repeated inquiries by this laboratory from manufacturers interested in the improvement of their product and a considerable reduction in the market price of aluminium sulphate, the matter was re-opened, and accurate experimental results were obtained.

Methods of Measuring Plasticity

Numerous efforts have been made to develop standard tests for plasticity or workability of hydrated lime; perhaps the best known is the Emley instrument which is much used in the United States. In the determination by means of this instrument, the "suction" feature of the backing predominates. The "working time," the period during which the putty remains coherent while it is being sheared on a standard porous backing, is measured. A disadvantage of this instrument from the point of view of development work lies in the fact

that considerable care is necessary in the preparation of the backings and a limited number of tests can be put through in the course of a working day. Another instrument which has considerable interest is a flow-table device developed by Mr. J. S. Cowper, Building Research Station, Carston, England. This apparatus is very simple in its action and was found to give such satisfactory results that it was used throughout the investigation reported here. It depends for its action on the spreading of a cone of putty when "knocked" on the platform of a standard flow table under specified conditions. The more plastic a putty the greater number of knocks required to spread it to a specified diameter. This very interesting characteristic presumably depends on the voids existing in the mass, coupled with the "cushioning" or shock-absorbing effect of the gelatinous surface layers.

For detailed specifications of the flow table the reader is referred to publications of the Building Research Station, Department of Scientific and Industrial Research, England.

The relation between the results obtained with the flow table and those obtained with the Emley instrument is not a linear one, but the following indicates the approximate degree to which the flow table shows the properties of a lime: poorly plastic limes, 12 to 14 knocks; limes of good quality for finishing purposes, 20 to 30 knocks; very highly plastic limes, more than 30 knocks. Limes requiring more than 30 knocks have a tendency to slump and, when in place, to remain soft for undesirably long periods, thus slowing up the finishing operation.

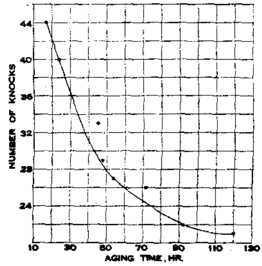


Fig. 1. *Effect of aging time on plasticity; lime No. 4598 (dolomitic). The two points that lie appreciably off the curve were obtained when a large excess of tempering water was used.*

Major changes in the plasticity of dolomitic or magnesian hydrates as indicated by this instrument take place when the material is aged in water. It is rather difficult to explain this satisfactorily, but in one case a relative workability at 24 hr., corresponding to 40 knocks, was reduced to about 21 knocks on aging 120 hr. out of contact with air. (Fig. 1 shows graphically this decrease.) It was therefore necessary to observe carefully the time factor in aging, particularly in the testing of magnesian hydrates. For the tests reported below, an aging period of 17 hr. was adopted as standard.

Experimental

As it was considered that appreciable proportions of aluminium sulphate would be necessary to give sufficient improvement in the properties of numerous limes, a comparison was made of the color given to a white lime by ordinary

commercial aluminium sulphate, $Al_2(SO_4)_3.18H_2O$, and that given by "iron-free" quality when both were mixed with nine parts of hydrated lime and tempered with water. No difference in color could be discerned, and it was concluded that the cheaper product could be used successfully. This material is available in large quantities for purposes of water purification and is usually purchased according to the following specification:

Not less than 17% water soluble alumina.
Not more than 0.5% total water insoluble material.
Not more than 0.75% Fe_2O_3.

The material is obtainable in the granular form.

In the preliminary trials of aluminium sulphate additions, the limes used were of high-calcium content, as shown by the chemical analyses given in Table I.

TABLE I

ANALYSES OF LIME SAMPLES

Lab. no. of sample	SiO_2	R_2O_3	CaO	MgO	H_2O	CO_2
4597	3.8	1.4	71.8	Trace	22.2	0.8
7300	1.2	0.6	72.0	1.3	23.7	1.3
7301	0.6	0.5	73.0	0.7	23.5	1.9

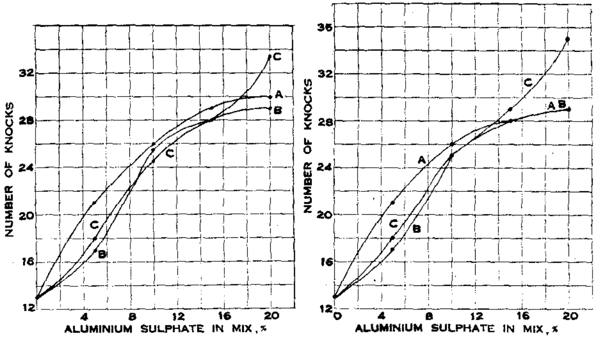
When tempered with water, aged, and brought to standard consistency, each of these samples required 13 knocks in the plasticity test, and was definitely inferior to finishing hydrate of acceptable quality. In treating them with aluminium sulphate the initial procedure followed was to dissolve a sufficient amount of the salt in rather less than the amount of water required for tempering, add this to the dry material and bring to the standard consistency with distilled water.

Figs. 2 and 3, Curve A, show quantitatively the effect of this addition; the results agree within one knock for the different limes. It will be observed that an aluminium sulphate content of 5–20% gives a lime equivalent in knock requirements to finishing hydrates of good quality.

As the only practical method of treating hydrated lime with aluminium sulphate would be to add it in the pulverized form, tests were made to determine the influence on the resulting plasticity of dry mixing of the aluminium sulphate. It was recognized that the material would have to be reasonably finely ground and no tests were made on sulphate coarser than 80 mesh.

Commercial aluminium sulphate is rather a difficult material to pulverize because it tends to decompose at higher temperatures and dissolve in its water of crystallization. This causes it to "gum" in pebble mills or disc pulverizers. Although no experiments have been made in this connection, there does not seem to be any reason why the sulphate could not be ground

successfully in an impact pulverizer, provided reasonable care were taken to avoid overheating. Furthermore, grinding in the presence of the required amount of lime gives ample protection against "gumming" or "balling." Tests were accordingly made of the effect on the properties of limes Nos. 4597, 7300, and 7301 of additions of aluminium sulphate both when ground separately to minus 80 mesh, and when mixed with the lime and ground to minus 100 mesh.



FIGS. 2 and 3. *Effect of addition of aluminium sulphate ($Al_2(SO_4)_3.18H_2O$) on plasticity.* FIG. 2. *Lime No. 7300 plus lime No. 7301, average of two determinations.* FIG. 3. *Lime No. 4597. In both figures—A, lime plus sulphate in solution; B, lime plus sulphate of minus 80 mesh; C, mixture of lime and sulphate ground to minus 100 mesh.*

Curves B and C, Figs. 2 and 3, show these results. They may be compared with those obtained by adding the aluminium sulphate in solution. It will be observed that the effect is comparable in all cases except when the lime and aluminium sulphate were pulverized together. In this instance the continued improvement in knock requirements is rather difficult to explain. The results, however, were duplicated in a number of tests. It is evident that for small amounts of aluminium sulphate, granted good mixing conditions, the best results are given when it is added in solution. This might have been anticipated: on the other hand, if carried out in practice it would leave the addition in the hands ot plasterers or helpers and permit considerable opportunity for error.

It was concluded from the above results that a mixture of 10% of aluminium sulphate and a lime of the type of No. 4597 would be a reasonable basis for practical trials of the treatment. Numerous factors must be considered, an important one being, of course, the increase of putty yield, which tends to diminish the cost per unit of wall area. On the other hand, the rate of change of the slope of the curve approaches a maximum at 10%, and with aluminium sulphate at $30.00 per ton and a hydrate cost of $8.00 per ton, the minimum

net increased sales value necessary to cover the material costs alone of a 10% addition would be of the order of $2.20 per ton. It was considered that any cost appreciably higher than this would remove any interest that a lime manufacturer might have.

In order to evaluate the foregoing results in a practical way, arrangements were made for the application of test panels of approximately 24 sq. ft. each in area by a well known local plastering contractor. Mixes of the three types described above were made up and compared with a standard finishing hydrate of high grade from the contractor's warehouse. No instructions were issued to the plasterer other than that he should use the sample according to his customary procedure.

The hydrate used was similar to the three mentioned above and had a workability corresponding to 13 knocks on the flow table. In each case the same proportion of gauging plaster and sand was added and a common backing of standard hardwall plaster was used. The results as observed during application and as subsequently reported by the plasterer through the contractor were as follows:

(a) Hydrate to which aluminium sulphate had been added in solution: This material, possibly owing to inexperience in the addition of large quantities of fairly strong aluminium sulphate solution, tended to be lumpy and was somewhat inferior to the standard magnesian finishing plaster.

(b) Hydrate with which aluminium sulphate was ground to minus 100 mesh: This material was also noticeably inferior to the magnesian hydrate. It showed a slight tendency to "pull" and break. This was probably due to the fine grinding which it had received in the disc pulverizer, for a similar result has been observed with other materials.

(c) Hydrate to which minus 80 mesh aluminium sulphate had been added: This material could not be distinguished from the high grade magnesian hydrate in its behavior under the trowel. The mechanic reported that he would not venture to state that it was at all inferior in "feel."

The four specimens were finished in the same way. All gave very excellent textures; the inferiority noted in the case of *(a)* and *(b)* was manifested only in the slight increase in work required. After a month's aging, no change could be observed in the appearance of the specimens; there was no evidence of the "bloom" which is reported to have been encountered when aluminium sulphate was added to quicklime before hydration.

As a final check on the accuracy with which the flow table indicates the effect of aluminium sulphate additions on the plasticity of the lime, the control magnesian hydrate used in the above-mentioned tests was tested on the flow table. Its rating, according to the standard method, was 25 knocks, which, it will be noted, is of the same order as that of the three mixtures of hydrate plus 10% of aluminium sulphate. It had evidently been duplicated in its properties by the high-calcium lime with addition of aluminium sulphate. It is significant to note that the cost of the magnesian hydrate in this territory is approximately $22.00 per ton.

There was no opportunity to make an accurate estimate of the covering power of these four hydrates during the practical test. The evidence apparently pointed to the magnesian hydrate as being somewhat superior to the others.

In order to have more precise information, "volumes in water" were measured for Sample 7316 plus 10% aluminium sulphate, Sample 7316 untreated, and Samples 4598 and 7315. The last two were high-priced magnesian hydrates. The results are given in Fig. 4 and show a much higher volume in water for the high-calcium lime treated with aluminium sulphate.

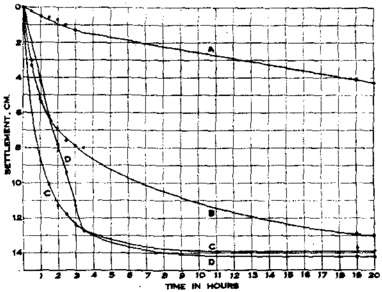


FIG. 4. *Volumes in water of various limes. A, lime No. 7316 plus 10% of aluminium sulphate; B, lime No. 4598; C, lime No. 7315; D, lime No. 7316.*

A further point worthy of note is the increased holding capacity for water which aluminium sulphate additions induce. This is shown in Fig. 5, the data for which were obtained at the same time as those on which Fig. 2 is based. It should be observed that, with more than 10% of aluminium sulphate added in solution, the putty tended to become "sloppy," and therefore undesirable from the point of view of application. Were it not for this fact it is probable that there would have been agreement between Curves A and C in Figs. 2 and 3.

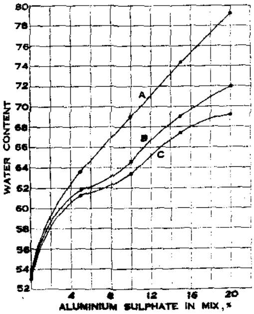


FIG. 5. *Effect of addition of aluminium sulphate on the water-holding capacity of lime (lime No. 4597). A, lime plus aluminium sulphate in solution; B, lime plus sulphate of minus 80 mesh; C, mixture of lime and sulphate ground to minus 100 mesh.*

No definite effect could be ascribed to the aluminium sulphate in relation to setting time or final hardness of the plaster. On the other hand, no deleterious effect is anticipated, as the amount of aluminium hydroxide present is considerably less than 2%. Actually, additions of aluminium hydroxide have been proposed as a means of increasing the final hardness of lime mixtures (5).

It would be reasonable to expect a certain amount of deterioration of lime-aluminium sulphate mixtures in storage, owing to slow reaction and

flocculation of the aluminium hydroxide under dry conditions. To investigate this point, tests were made on mixtures when freshly prepared and after aging in the dry state for a three-week period. The results were as shown in Table II.

TABLE II

DETERIORATION OF MIXTURE DURING STORAGE

Aluminium sulphate mixture		10%	20%
Knock requirement:	Initial	25	33
	After storage	23	30

These results indicate that hydrates containing 10% of aluminium sulphate might be stored for reasonable periods without serious deterioration.

It is considered that these results justify extended commercial trials on the part of certain lime manufacturers whose main tonnage consists of chemical lime or of high-calcium hydrate at present unsuitable for finishing purposes. No great difficulty is anticipated in the addition of the aluminium sulphate, for if it were fed at the pulverizer, adequate mixing without excessive grinding would be assured.

Acknowledgment

The authors wish to acknowledge the co-operation of Mr. M. F. Goudge, of the Department of Mines, Ottawa, who supplied data on plasticity tests with the Emley instrument.

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NOTE ON AN OPTICAL SYSTEM FOR SHADOW RECORDING WITH OSCILLOGRAPHS[1]

BY G. A. WOONTON[2] AND F. W. PYE[3]

Abstract

In this paper is discussed a method of shadow recording for use with reflecting types of oscillographs which simplifies the methods of obtaining time and amplitude rulings on the record. The optical system is rendered portable by the use of a low-intensity light source, compensated by high transmission efficiency. Comparative records indicate that, when used in conjunction with bromide paper, this optical system is of value in recording sinusoidal currents whose frequencies are below 20 cycles per second or currents of complicated wave form whose fundamental frequency is well below 20 cycles per second. No data relative to records on moving film are reported.

Mann (1), in discussing a new portable electrocardiograph, has referred to an optical system of this type. The authors believe that the present paper contains sufficient new information on the details of construction and operation of this optical system to make it of value.

Methods of Oscillograph Recording

Oscillograph records on a moving film or strip of bromide paper are made either with a moving spot or line of light on a dark field, or a moving line shadow on a bright field, depending on the type of oscillograph used. The record is of value for the analysis of the varying currents only when the units (usually e.m.f. against time, or current against time) against which it is plotted, are known, preferably from markings carried on the record itself. Unit markings can be superimposed on the record by periodically varying the light intensity to which the whole film is exposed, both in extension and time; the complexity of the apparatus necessary to bring about such periodic variations in intensity varies with the recording system used.

The bright line or spot method lends itself to recording when the oscillograph element is of the reflecting type. Fig. 1 is a record of a sinusoidal current obtained in this manner.

This method is wasteful of light flux. If a line of light is used, the line must be generated either by a slit or a cylindrical lens. In the first case all but the small fraction of light flux which passes through the slit is screened out; in the second (since it is not generally convenient to place a cylindrical lens in front of the oscillograph mirror), a broad patch of light floods the mirror plane, but only that portion which is reflected by the mirror reaches the camera. Similar conditions obtain when recording with a spot of light. Intense light sources are required in all cases.

Amplitude rulings are placed on the record by flooding the paper with light of low intensity from a second source, through a cylindrical lens which

[1] *Manuscript received November 22, 1934.*
Contribution from the Department of Physics, University of Western Ontario, London, Ontario, Canada.
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[3] *Instrument Maker, Department of Physics, University of Western Ontario.*

has been etched with millimetre marks in the slit of the camera. Time rulings may be recorded by interrupting the flood lighting beam periodically with a rotating vane, or by allowing a high-intensity beam from a third source to flash periodically on the camera slit, through holes in a rotating disc. Fig. 1 shows amplitude rulings recorded by this method.

The shadow method of recording was originally developed for use with the Einthoven string galvanometer. An Einthoven record obtained in this manner is shown in Fig. 2. A beam of light is passed through the galvanometer in such a manner that a circular patch of light, across which the shadow of the string moves, is developed in the plane of the camera slit. Amplitude rulings on the camera lens furnish recorded amplitude units. A rotating vane interrupts the recording beam periodically and produces time rulings on the paper. One simple optical system and one light source perform the function of the two or three sources and systems required by the other method.

Shadow Recording with Reflecting Oscillographs

Fig. 3 is a schematic diagram of an optical system developed by the authors to make possible the application of the Einthoven type of recording to reflecting oscillographs. Figs. 4*a* and 4*b* are photographs of the optical system in a portable form suitable for general laboratory use.

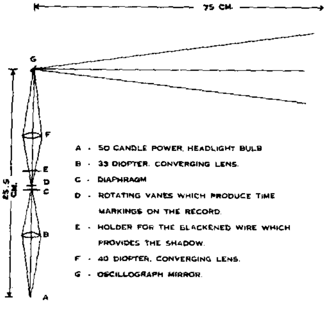

FIG. 3. *Schematic diagram of the optical system.*

The image of the hot filament of a 50 candle power head light bulb is focused on the mirror of an oscillograph by a system of two lenses. The reflected rays diverge from the oscillograph mirror to form a circular spot of light whose diameter is about twice the length of the camera slit. A hairline of blackened wire is located between the two lenses in such a position that a sharp image of the hairline is formed in the plane of the camera slit. The movement of the oscillograph mirror deflects both the image of the hairline and the light spot, at the camera, but since the spot of light is twice as wide as the slit, the slit is always flooded with light regardless of the motion of the hairline.

Amplitude rulings are recorded by the ruled cylindrical lens method. Timing lines are recorded by cutting the light beam periodically at the focal point *D*, by a set of rotating vanes driven by a synchronous motor. In this case the motor carries five vanes, equally spaced, and rotates five times per second. The markings are therefore spaced at intervals of $\frac{1}{25}$ sec. A heavier

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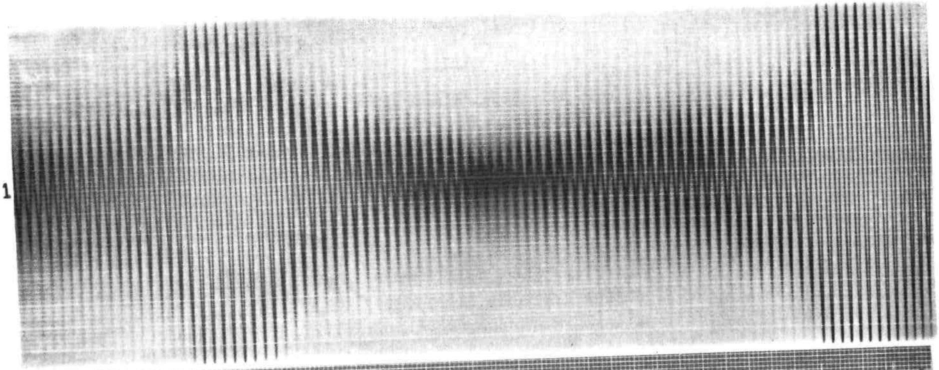

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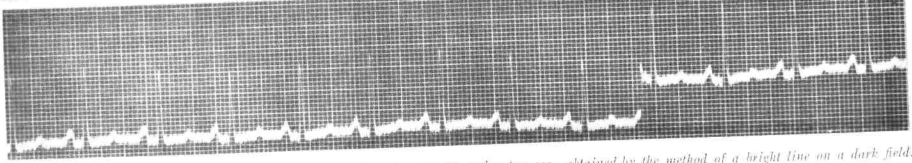
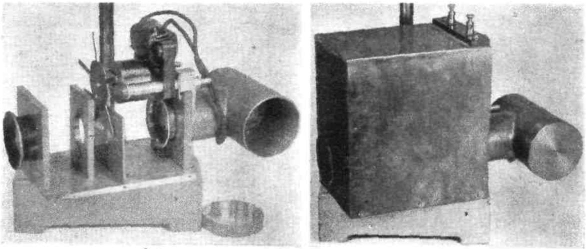


FIG. 1. *Record of a sinusoidal e.m.f., frequency approximately 20 cycles per sec., obtained by the method of a bright line on a dark field.*
FIG. 2. *Cardiogram recorded by the shadow method with an Einthoven galvanometer.*

fifth-second line is obtained by making one of the five vanes wider than the others. A diaphragm at *C*, just behind the rotating vanes, cuts down stray light (due to aberration and other causes) which tends to decrease the contrast of the time graphing.



4a 4b

FIG. 4. (a) *The complete optical system with covers removed.* (b) *The optical system with covers in place.*

Frequency Limit of Usefulness

Figs. 5*a*, *b*, *c*, are records made with this system of currents whose frequencies were approximately 4, 10 and 20 cycles per second. Fig. 6 is a cardiogram recorded with a reflecting oscillograph and this system, but without the timing equipment in operation. On the basis of these results it is believed that the system is of value in recording sinusoidal disturbances whose frequencies do not exceed 20 cycles per second, and all types of disturbances whose fundamental is well below 20 cycles per second, even though relatively high harmonics are involved. These data are for contrast grades of bromide paper; no data are at hand regarding the limitations on the system when recording with film.

Precautions

The system discussed above has been designed to operate with a distance of less than 10 cm. between oscillograph mirror and front lens, in order to take full advantage of the possibilities of efficiency and portability associated with the high power lenses used. When the oscillograph is used under conditions of extreme sensitivity and low-frequency response it occasionally picks up a ripple from the magnetic field of the synchronous motor. Magnetic shielding of the optical system or oscillograph will eliminate this source of interference.

The records shown here were made with an optical system whose lenses were not corrected for chromatic aberration; with such lenses the records obtained are sometimes poor. This source of trouble is eliminated by the substitution of achromatic doublets, but their use adds materially to the cost of the system.

If the headlight bulb is operated from an alternating current source, precautions must be taken to prevent synchronization of the vane position with filament current. Synchronization of this kind will cause indistinctness over part, or all, of some of the time marks.

Irregularities in the camera paper speed (such as that caused by a metal rivet in the camera drive belt), give rise on the record to lines that are easily confused with time lines. These ambiguities are avoided by the use of a sewn belt or gears to drive the camera.

Acknowledgment

The writers wish to thank President W. Sherwood Fox and Messrs. Gordon Ingram and A. E. Silverwood of the Board of Governors of this University for financial and other assistance, which has permitted completion of this work as part of a general program intended to facilitate the use of physical equipment in medical research.

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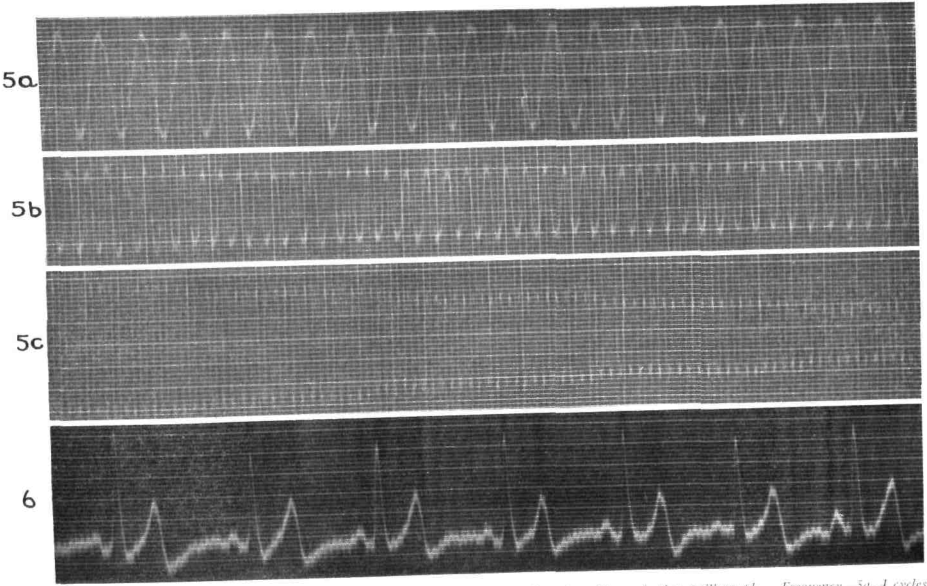

FIGS. 5a, b, c. *Sinusoidal e.m.f.'s recorded by the shadow method in conjunction with a reflecting oscillograph. Frequency—5a, 4 cycles per sec.; 5b, 10 cycles per sec.; 5c, 20 cycles per sec.* FIG. 6. *Cardiogram recorded by the shadow method with a reflecting type of oscillograph.*

MAGNETIC EFFECTS IN SUPERCONDUCTORS[1]

By F. G. A. Tarr[2] and J. O. Wilhelm[3]

Abstract

This paper deals with magnetic effects in metals cooled from above the transition temperature to below the superconducting temperature, while in an applied magnetic field. Previous work of Meissner and Ochsenfeld indicates that at the superconducting temperature the effective permeability of the metal became zero. It is found, however, that the effective permeability is greatly influenced by the composition and geometrical shape of the specimen. There is also, in general, a reduction in flux on removing the magnetic field after the superconducting temperature has been reached, but on re-establishing the field the flux through the specimen remains unchanged.

Introduction

Since the discovery, by Meissner and Ochsenfeld (1), of the permeability changes taking place in a superconductor when it is cooled in a magnetic field, the writers have carried out several experiments with a view to finding out how the results are affected by the composition and geometrical shape of the superconductor. Although this work is not yet complete it seems worth while, at this stage, to collect and present the results so far obtained. The earlier experiments were designed with a view to finding whether there was a limiting particle size below which superconductors would fail to show any change in effective permeability on being cooled from above the transition point down to the superconducting state while in a constant magnetic field. Other experiments on the effect of shape and composition of the specimen suggested themselves as the investigation proceeded.

Experiments

As the work, so far, has not called for any great accuracy, measurements were made using a fluxmeter with stationary search coils as described in a previous paper (3). The magnetic field was produced by two circular coils so arranged, on opposite sides of the cryostat, as to give an approximately parallel field in the region of the specimen under test.

The essential tests made on each superconducting specimen were:—

(a) The determination of the reduction in flux passing through the specimen as it was cooled, in a constant magnetic field, from above the transition temperature to below the superconducting temperature corresponding to the field strength used.

(b) The determination of the reduction in flux passing through the superconductor on removing the external magnetic field directly after process *(a)* had been carried out. Hereafter, for brevity, Test *(a)* will be referred to as *reduction in flux on cooling* and Test *(b)* will be referred to as *reduction in flux on removing field.*

[1] *Manuscript received September 14, 1934.*
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In many of the specimens tested the search coil windings were not completely shielded by the superconductor which they enclosed, so that when a magnetic field was applied with the specimen at, or below, the superconducting temperature, corresponding to the field strength used, some flux still linked the search coil as indicated by the fluxmeter deflection, and this was taken into account as seen below.

Let T_n be a temperature at which the specimen is completely non-superconducting and T_s be a temperature at which the specimen is completely superconducting for the particular field strength used. If, with the specimen at temperature T_n, an external field is applied, the fluxmeter deflection obtained corresponds to the total flux, ϕ, threading the search coil. Now $\phi = \phi_s + \phi_c$, where ϕ_s is the flux threading the exposed part of the search coil only, and ϕ_c the flux threading the conductor within the search coil. If the specimen is now cooled from T_n to T_s, with no external field applied, and then the same field as above is applied, the deflection obtained corresponds to ϕ_s alone, as no flux can pass through the superconductor in this state. Thus ϕ_c can be calculated. With the specimen again at T_n and the same external field applied, the specimen is now cooled to T_s. The fluxmeter deflection then gives the reduction in flux, ϕ_{rc}, passing through the superconductor. Now, with the specimen still at temperature T_s, the external magnetic field is removed and the deflection gives the reduction in flux, ϕ_r, threading both the superconductor and the exposed part of the search coil. Then $\phi_r = \phi_{rf} + \phi_s$, where ϕ_{rf} is the reduction in flux through the superconductor, and ϕ_s, as before, is the part of the external field threading the exposed part of the search coil only, obtained as outlined above. Thus the reduction in flux in the superconductor, ϕ_{rf}, can be calculated.

For convenience the results are given in percentages as follows:—

Percentage unshielded flux

$$= \frac{\phi_s}{\phi} \times 100\%$$

Percentage shielding

$$= \frac{\phi - \phi_s}{\phi} \times 100\%$$

$$= 100\% - \text{percentage unshielded flux}$$

Percentage reduction in flux on cooling

$$\text{Observed} = \frac{\phi_{rc}}{\phi} \times 100\%$$

$$\text{Corrected} = \frac{\phi_{rc}}{\phi_c} \times 100\% = \left(\frac{\phi_{rc}}{\phi} \Big/ \frac{\phi - \phi_s}{\phi}\right) \times 100\%$$

$$= \frac{\text{Observed percentage reduction in flux on cooling}}{\text{Percentage shielding}}$$

Percentage reduction in flux on removing field

$$\text{Observed} = \frac{\phi_r}{\phi} \times 100\%$$

$$\text{Corrected} = \frac{\phi_{rf}}{\phi_c} \times 100\% = \left(\frac{\phi_r - \phi_s}{\phi} \Big/ \frac{\phi - \phi_s}{\phi}\right) \times 100\%$$

$$= \frac{\text{Observed percentage reduction in flux on removing field} - \text{percentage unshielded flux}}{\text{Percentage shielding}}$$

Of course, corrections made in this way are not strictly accurate (except where all the flux through the superconductor disappears completely on cooling), as it assumes the flux through the unshielded part of the search coil to be constant whereas it actually varies, being greater the less the effective permeability of the superconductor. However, the accuracy of the work did not warrant attempting any more accurate corrections.

In cases where a reduction in flux was found on removing the external magnetic field, as in the above process, it was found that on immediately reapplying the field the deflection of the fluxmeter was exactly the same as if the specimen had been cooled with no external field to the superconducting temperature and a field then applied, *i.e.*, the fluxmeter deflection corresponded to the percentage unshielded flux. In other words this process was not reversible.

Below is given a description of the various specimens tested. The drawings indicate, in addition to the sizes, the positions of the search coils and directions of the applied fields. The field intensities used ranged from 50 to 150 gauss. All specimens were generally of cylindrical shape, either elongated or disc-shaped. In some cases axial, and in others transverse, fields were applied, as indicated by the arrows in the figures.

No. 1. A hollow cylinder of commercial block tin (Fig. 1a).

No. 2. An emulsion of mercury and lard, approximately 50% mercury–50% lard by volume, contained in a glass vessel (Fig. 5). The size of the mercury particles was of the order of 10^{-4} cm. diameter; they appeared under a high power microscope to be surprisingly regular. The emulsion was produced by grinding the mercury into the lard, melting the whole, and then centrifuging while hot.

No. 3. Three pulley-shaped specimens (Fig. 7) made of lead-tin alloy. One was a eutectic (37% lead–63% tin). The other two varied in composition, one being rich in lead (60% lead–40% tin) and the other rich in tin (20% lead–80% tin). All gave the same results.

No. 4. Rose metal (50% bismuth–27.1% lead–22.9% tin) cast round search coil (Fig. 8).

No. 5. Small quartz pebbles embedded in mercury contained in a glass vessel (Fig. 5). Approximately 50% mercury–50% quartz by volume.

No. 6. A solid cylinder of commercial block tin (Fig. 4).

No. 7. A pulley-shaped specimen of commercial block tin (Fig. 7).

No. 8. A pulley-shaped specimen of commercial lead (Fig. 7).

No. 9. Mercury contained in a glass vessel (Fig. 5).

No. 10. A hollow lead cylinder (Fig. 1b).

No. 11. A pulley-shaped specimen of tantalum (Fig. 7).

No. 12. A solid cylinder of commercial block tin (Fig. 6). The search coil was wound in a groove in the cylinder as shown and covered with a strip of tin soldered (with tin) to the cylinder.

No. 13. A solid thin cylinder of commercial block tin (Fig. 2a).

No. 14. A solid thick cylinder of commercial block tin (Fig. 2b).

No. 15. A hollow cylinder of commercial block tin (Fig. 3).

The results of the experiments on these specimens are given in Table I.

TABLE I

MAGNETIC REACTIONS OF SUPERCONDUCTING SPECIMENS

Specimen No.	Fig.	Description	Percentage unshielded flux	Percentage *shielding*	Percentage reduction in flux on *cooling*		Percentage reduction in flux on *removing field*	
					Obs.	Corr.	Obs.	Corr.
1	1a	Hollow tin cylinder	15	85	35	41	—	—
2	5	Mercury and lard emulsion	71	29	29	100	0	0
3	7	Tin-lead alloy pulleys	3	97	0	0	3	0
4	8	Rose metal	0	100	0	0	0	0
5	5	Quartz embedded in mercury	0	100	20	20	30	30
6	4	Solid tin cylinder	12	88	25	28	16	5
7	7	Tin pulley	3	97	10	10	40	38
8	7	Lead pulley	5	95	11	12	29	25
9	5	Mercury	0	100	85	85	15	15
10	1b	Hollow lead cylinder	8	92	60	65	8	0
11	7	Tantalum pulley	7	93	1	1	10	3
12	6	Solid tin cylinder (embedded coil)	0	100	65	65	16	16
13	2a	Solid tin cylinder (thin)	19	81	34	42	45	32
14	2b	Solid tin cylinder (thick)	12	88	24	27	65	60
15	3	Hollow tin cylinder Coil 1	9	91	24	26	24	16
		Hollow tin cylinder Coil 2	0	100	−23	−23	0	0

In most cases repeated tests on the various specimens checked within the limits of experimental error. There was one striking exception, however, in the case of lead pulley (No. 8), where the discrepancies were far beyond possible experimental error. For this experiment, in Table I, are given the average values obtained; the actual corrected values for these tests were 3, 10 and 20% reduction in flux on cooling, and 15, 21 and 39%, respectively, reduction in flux on removing the field. These discrepancies were thought to be due to the way in which the specimen was cooled. As lead becomes

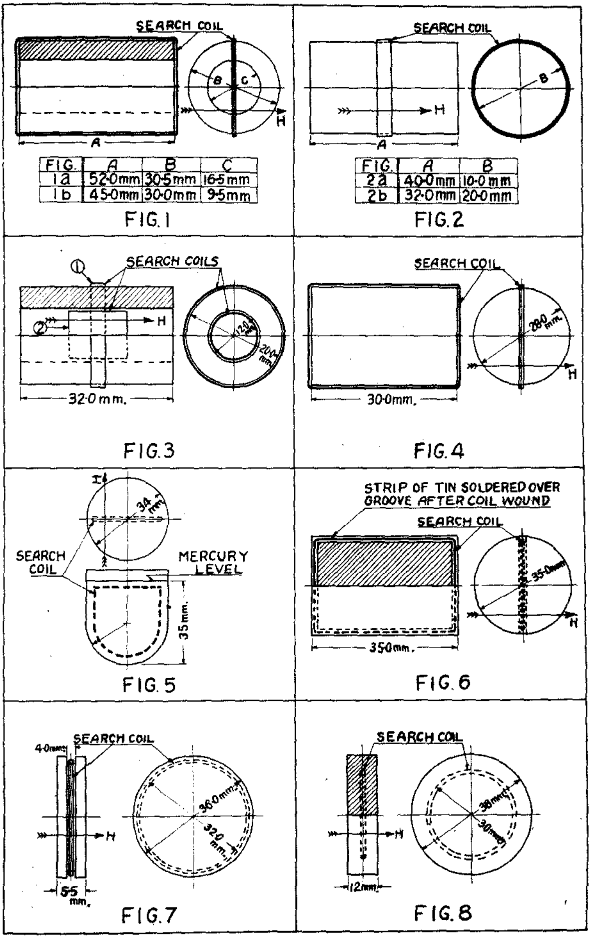

FIG.	A	B	C
1a	52·0mm	30·5mm	16·5mm
1b	45·0mm	30·0mm	9·5mm

FIG.	A	B
2a	40·0mm	10·0mm
2b	32·0mm	20·0mm

FIGS. 1-8. *Details of specimens referred to in Table I.*

superconducting for the field strengths used above the temperature of liquid helium, the cooling was necessarily carried out in a stream of cold helium gas, so that it was probable that the specimen was not cooled uniformly in each case. In an endeavor to find how the manner in which cooling was carried out affected the results, the hollow lead cylinder (No. 10) was, by the aid of suitable insulation, tested with cooling taking place from either the inside or the outside. In both cases readings of reduction in flux on cooling and on removing the field were taken but no difference was observed.

The only case in which flux passing through the superconductor was completely removed on cooling was that of the mercury and lard emulsion, *i.e.*, the effective permeability of the mercury particles became zero. It will also be seen that only with mercury (No. 9) was the sum of the reductions in flux on cooling and removing the field 100%. The alloys, on the other hand, showed no reduction in flux either on cooling or removing the field.

In addition to the above experiments hysteresis curves were plotted for several of the specimens. Only one curve (Fig. 9) is given as being representative, that of the solid tin cylinder with embedded search coil (No. 12). To obtain this curve the specimen was cooled with no external field to some temperature below the superconducting temperature, but not so low that superconductivity could not be destroyed by the magnetic field at the writers' disposal. The temperature was then kept constant and a magnetic field applied and increased step by step, the fluxmeter reading being noted at each step. After a field sufficient to destroy superconductivity had been applied, the field was lowered step by step, the fluxmeter being read, as before, at each step. The arrows on the curve (Fig. 9) indicate the direction of increasing and decreasing applied field. The curve is plotted to an arbitrary scale. No great accuracy is claimed for this curve as, owing to the time taken, the fluxmeter drift was quite appreciable and corrections had to be made for this.

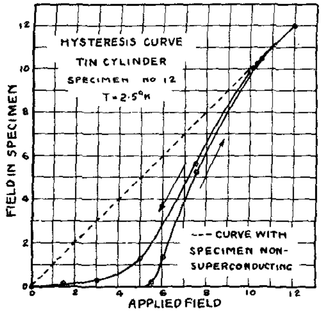

FIG. 9. *Hysteresis curve of a superconductor.*

Conclusion

The original experiments of Meissner and Ochsenfeld indicated that when a superconductor was cooled, in a constant magnetic field, from above the transition temperature to below the superconducting temperature, the flux in the superconductor was either almost or completely annulled. The writers'

experiments show that in general this is not the case, and that there are wide variations in the amount of flux reduction, depending on the shape and composition of the superconductor and on the direction of the applied field. In this the results agree with the result obtained by Mendelssohn and Babbitt in one of their experiments (2) in which they cooled a tin sphere in a constant magnetic field and found, after removing the field, that the sphere had a magnetic moment, although this was hardly to be expected in view of the previous work of Meissner and Ochsenfeld.

Acknowledgment

The authors wish to thank Professor E. F. Burton, Director of this Laboratory, for his encouragement in this work, and also Dr. H. Grayson Smith and Dr. C. Barnes for suggesting several of the experiments recorded herein.

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REVIEWS AND NOTES

On a Method of Viewing and Recording Induction Coil Transients with the Cathode-ray Oscillograph

Transient phenomena, in general, cannot be scanned on the cathode-ray oscillograph screen by means of the standard sweep oscillator, because of the non-recurrent nature of the wave and the high velocity of the spot. If the transient is periodic, that part of each wave train which supplies the synchronizing pulse is lost.

The writer's immediate problem lay in the development of apparatus to generate the timing voltage for a cathode-ray oscillograph and in the synchronization of this voltage wave with transients produced by induction coils under the conditions maintained by physiologists when stimulating nervous tissue.

Two methods of stimulation are in common use. Single-shock stimulation involves the production of a single transient in the secondary circuit by a single make or a single break of the primary circuit. Faradic stimulation is produced by periodic transients, generated by periodically interrupting the primary current of the coil either by means of a vibrator or a rotary commutator, which is often fitted with a second set of contacts by which either the make or the break shock may be suppressed by short circuiting the secondary at the appropriate moment. The synchronizing device must be sufficiently flexible to allow retracing of the pattern for primary interruption periods long enough to allow a single transient to be produced and die out completely, and short enough to allow several transients to be partly superimposed if this case ever occurs in practice.

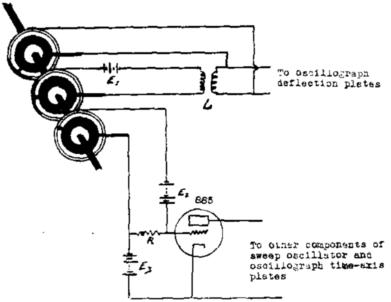
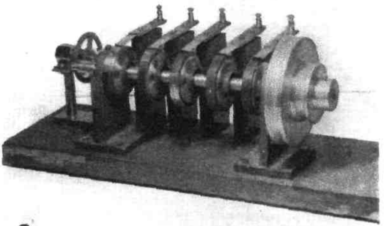


FIG. 1. *Diagram of connections.* E_1—*Primary battery for coil under observation.* E_2—*Battery to supply synchronizing pulse.* E_3—*Grid-glow tube bias battery.* L—*Coil under observation.* R—*Coupling resistor in the grid circuit of the grid-glow tube (this resistor may be replaced by a transformer with secondary coupled into the grid circuit).*

Methods involving relays and more complicated equipment were eliminated for the reasons given above and also because of false transients due to bad arcing at the relay contacts.

Fig. 1 illustrates the circuit and associated equipment developed to overcome these difficulties. It is essentially a combination of the methods of

McFarlane (1) and Turner (2) applied to a sweep oscillator. Fig. 2 is a reproduction of a rotary commutator much like those employed by physiologists and equipped with a synchronizing wheel and contact in addition to those usually employed for making and breaking the primary current and



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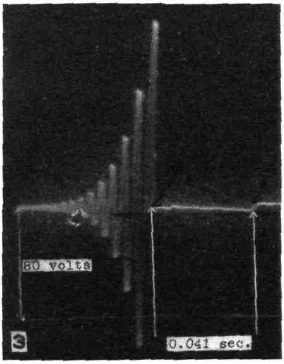

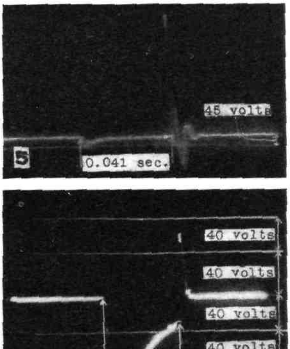

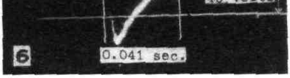

FIG. 2. *The synchronizing commutator. The revolution counter, used in calculating time intervals, is shown geared to the commutator at the left of the photograph.*

FIGS. 3, 4, 6. *Oscillograms obtained from a standard physiological stimulation coil, modified by shunting the interrupter with a condenser, capacity 1 μf.* FIG. 3. *Primary peak current 1.3 amp., iron core, secondary shunted by 75,000 ohms and moved out 10 cm. from position of closest coupling.* FIG. 4. *All conditions as in Fig. 3, except that the secondary was moved out an additional 15 cm.* FIG. 6. *Conditions as in Fig. 3, except secondary in position of closest coupling.*

FIG. 5. *Oscillogram obtained from a standard physiological stimulation coil. In this case the interrupter was not shunted by a condenser. Primary peak current 1.3 amp. iron core, secondary shunted by 1.6 megohms and in closest coupled position.*

suppressing undesired transients in the secondary circuit. The synchronizing wheel presents a bakelite surface to the brush which plays on its periphery except for one very narrow brass contact which occupies only a few minutes of arc of the entire circumference. This strip of brass is connected to a brass disc mounted on the side of the wheel on which a second brush plays. If the two brushes are placed in series with a circuit, the circuit is completed during a very small fraction of a second once in each revolution.

The grid of the grid-glow tube in the sweep oscillator is biased sufficiently negatively relative to the cathode that a discharge cannot take place unless a change in circuit constants occurs. The transformer or coupling resistor in the grid circuit of this tube is coupled in series with an external battery and the brushes of the synchronizing wheel in such a way that the external battery furnishes a pulse which neutralizes the negative bias on the tube, and allows a discharge to take place each time that contact is made at the synchronizing wheel. After each discharge the grid of the tube again takes control, and since the synchronizing contact has been broken as the commutator turns, a second oscillation cannot occur until the contact has completed one revolution and returned to its previous position. Since by this means the transient appears at a definite time after the spot starts its sweep across the screen, perfect retracing is possible. The transient wave may be spread across the screen, or condensed to any degree since the velocity of the spot is entirely independent of the periodicity of the sweep. Amplitude and frequency of the sweep oscillator are controlled in the usual manner. Figs. 3, 4, 5 and 6 are photographs of wave patterns produced on the oscillograph screen by induction coil discharges, controlled by the method just discussed.

Graphical analyses of transient patterns require a determination of the units on both the time and voltage axis. Voltage units may be easily determined by applying known voltages to the deflecting plates after the transient record is made. The exposed film shows these further records as graph lines. Some experimenters have obtained time units by superimposing a sine wave of known frequency on the record. The writer has found it more convenient to calculate the time interval between the start of the make and break transients directly from the film by means of data regarding the angular velocity of the commutator, furnished by a permanently attached revolution counter, and the known angular separation between two ends of the primary contact segment. In cases where either the make or the break transient is to be viewed alone, an extra contact wheel is used to supply a peak on the screen at a definite number of degrees of arc before the transient appears. As before, data sufficient to determine the time scale completely can be obtained from the film by a simple measurement of separation between the two impulses.

The various contact wheels of the commutator are each held in place by a single set screw. The position of the pattern on the screen can be readily adjusted by changing the angular position of the various contacts relative to one another. Allowance can also be made, by the same method, for phase changes if the e.m.f. under investigation is to be amplified or attenuated.

In the first experiments with this device, difficulties in synchronizing were discovered owing to impulses induced by the coils in the synchronizing network. It was found that these difficulties could be avoided by biasing the grid-glow tube far beyond the threshold of ignition reached by these false impulses, and counterbalancing the high bias (sometimes 90 volts) by a correspondingly high-potential battery in the synchronizing circuit.

The writer wishes to express his thanks to Professor F. R. Miller for suggesting this problem and for his help and suggestions regarding the physiological problems involved, and to Mr. F. W. Pye for his help in designing and constructing the commutator.

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THE DIFFERENTIAL BLOOD CHANGES IN ASCARIASIS IN FOXES[1]

BY ARNOLD H. KENNEDY[2] AND RONALD G. LAW[3]

Abstract

Definite and characteristic changes occurred in the proportions of basophiles, neutrophiles and lymphocyte cells in the blood of foxes which had received *Ascaris* eggs. The numbers of red blood cells and the amounts of haemoglobin remained within normal limits but the proportion of basophiles rose as high as 69.5% of the total leucocyte count. This increase is apparently associated with the number of eggs given; the largest dose producing the highest proportions. The proportions of neutrophiles and leucocytes tended to fluctuate in opposite directions. An increase in the total numbers of white blood cells appears to be influenced by an increase in the proportion of basophiles.

The cellular reaction of the blood to parasite infections indicates a definite attempt on the part of the body tissues to create a defence against them. The experiments described below were carried out in order to record the reaction of the various blood cells to *Ascaris* infection in foxes.

Experiments

Eight foxes, 10 to 12 weeks of age, giving a negative examination for one month by the D.C.F. technique, were infected with embryonated eggs from *Toxascaris leonina*. These and two control foxes were kept under identical conditions. The embryonated eggs were given orally in a small quantity of water by means of the stomach tube. The eggs were given to individual foxes in numbers approximating 500, 1,000 and 2,000. In one case, two doses of approximately 500 eggs were administered ten days apart, in order to record the cellular reactions when two small doses of eggs were given at a ten-day interval. After the eggs were administered the foxes were placed in individual wire cages so constructed as to allow the feces to pass through the wire to a drawer inserted some ten inches below. Previous to the administration of the eggs the blood of each fox was examined for normality on three occasions at intervals of three days. The standard for normal blood in foxes used in the investigation was that established by Kennedy (1).

[1] *Original manuscript received July 4, 1934.*
Contribution from the Ontario Government Experimental Fur Farm, Kirkfield, Ontario.
[2] *Veterinary Pathologist.*
[3] *Director.*

Staining Reactions of Foxes' Blood.

According to Kennedy (1) the eosinophile is not found in foxes' blood, but is replaced by a cell which is distinctly basophilic in staining reaction and which is classified as a basophile. Consequently, where investigations involving a rise in numbers of such cells are undertaken with foxes, eosinophiles are replaced by basophiles.

The blood studies show the total number of red blood cells and leucocytes, the differential leucocyte count and the number of grams of haemoglobin per 100 cc. of blood. These counts were made on each fox at three-day intervals. The cell counts were estimated with a standard haemocytometer and the haemoglobin readings by a Sahli haemoglobinometer. The blood smears for the differential blood count were stained with Hasting's stain, and two hundred leucocytes differentiated under the oil immersion lens. In all cases the foxes were bled in the morning, previous to feeding. Standardized pipettes were used throughout the study.

Red Blood Cells and Haemoglobin

As stated above, the ten foxes, previous to infection, were all within the normal range of haemoglobin and of red blood cells per cu. mm. At no time during the experiment was there any trend towards abnormality in either red blood cells or haemoglobin. During the experiment the red blood count ranged from seven to twelve millions per cu. mm. of blood, the numbers increasing toward the conclusion of the experiment while the haemoglobin ranged from 11.5 to 12.9 gm. per 100 cc. of blood. These figures are within the limits established for the blood of normal foxes.

Total Number of White Blood Cells

In all of the foxes given embryonated eggs definite and characteristic changes in the proportions of basophile, neutrophile and lymphocyte cells occurred. The fluctuations of the basophiles and of the total numbers of white blood cells per cu. mm. appear very similar. The total number of white blood cells tends to increase or decrease according to the proportions of basophiles present in the blood stream. When the proportion of basophiles was within normal limits the total numbers of white blood cells were found to be normal also. Apparently the proportions of the various white blood cells are affected by *Ascaris* infection, the most striking change being shown in the proportion of basophiles.

In Figs. 1-10 data from eight infected foxes and two controls are represented. In view of the similarity found in the counts of foxes receiving approximately the same number of *Ascaris* eggs, the graphs show the means for foxes given similar doses.

Each graph was prepared from the data obtained on the total numbers of red and white cells per cu. mm. of blood. The proportions of the basophile, neutrophile and lymphocyte cells were also recorded at various dates throughout the experiment. The proportions of monocytes were so low that they were not plotted. The abscissas show the dates of the examination, while

the ordinates show the proportions or, in some cases, the numbers of basophiles, neutrophiles and lymphocytes found in the blood. The relations of the proportions of these three classes of leucocytes, as seen in foxes that have been given varying quantities of embryonated *Ascaris* eggs, can readily be studied from these graphs.

Control Foxes G-31 and G-20 (Figs. 1 and 2)

The proportion of basophiles fluctuated from 2 to 7%, which is within the limits for normal foxes' blood. The general trend of the neutrophiles and lymphocytes was very similar to that of the basophiles, both being considered within the normal range.

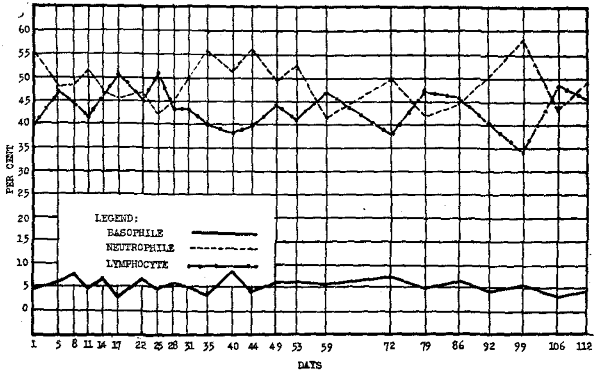

FIG. 1.

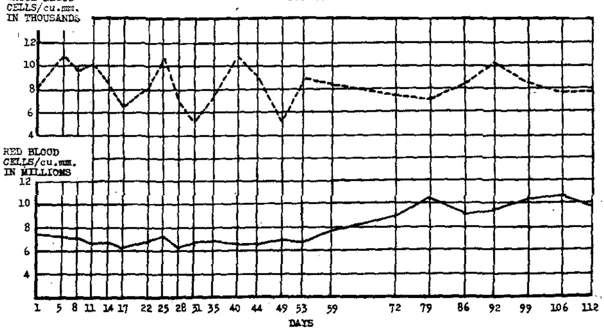

FIG. 2.

Foxes G-21 and G-23 (Figs. 3 and 4)

These foxes received approximately 500 embryonated *Ascaris* eggs. The proportion of basophiles reached the high point of 26% at 11 and 22 days after infection. After reaching the peak a decrease took place, the low point of 6.5% being reached on the thirty-ninth day. On the forty-eighth day an increase to 12.5% took place. An increase to 13.5% took place on the fifty-eighth day, after which the trend was downwards, the proportion of basophiles ultimately returning to normal.

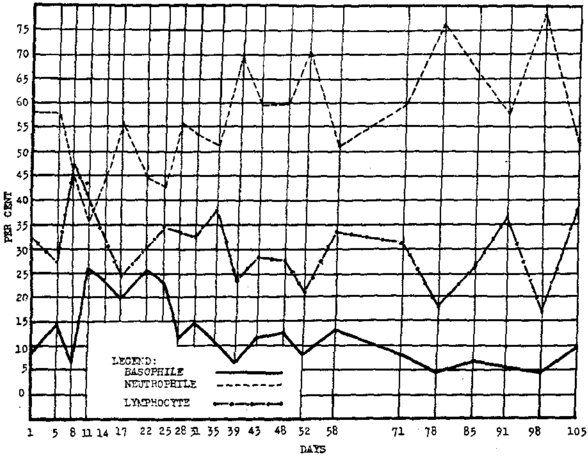

FIG. 3.

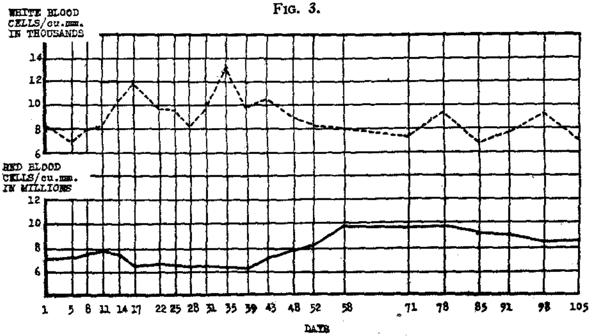

FIG. 4.

Foxes G-29 and G-30 (Figs. 5 and 6)

Approximately 1,000 eggs were administered, and four days after infection the proportion of basophiles and the total numbers of white blood cells commenced to increase. Fourteen days after infection the proportion of basophiles reached a peak of 59.0% and the total number of white blood cells

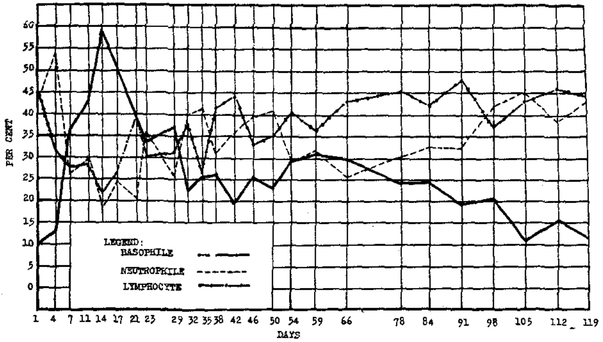

FIG. 5.

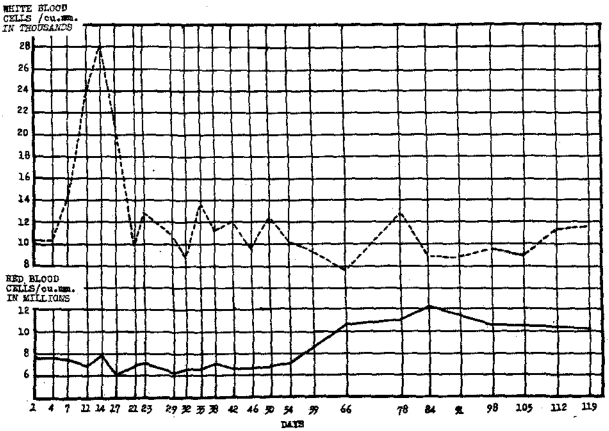

FIG. 6.

reached a peak of 28,000 per cu. mm. of blood. On the thirty-second day the proportion of basophiles dropped to 22.5% and the total number of white blood cells to 8,900 per cu. mm. of blood. After this date the trends were more or less level, the basophile proportions varying from 19 to 31% and the total number of white blood cells from 8,600 to 13,700, until the ninety-first day, when a decrease occurred. On the one hundred and fifth day the proportion of basophiles had diminished to 11%. After infestation the proportions of neutrophiles and lymphocytes decreased, the proportion of neutrophiles dropping from 44% to 18.5%, and the proportion of lymphocytes from 47 to 22%, fourteen days after infestation. After this date the general trend of both neutrophiles and lymphocytes was upwards but their daily fluctuations were in opposite directions. In these foxes, the total number of white blood cells and the proportions of lymphocytes and basophiles had similar trends. With an increase in the total number of white blood cells, the proportion of basophiles also tends to increase. With an increase in the proportion of basophiles, the proportion of lymphocytes also increases over the proportion of neutrophiles and when the proportion of basophiles decreases the proportion of neutrophiles increases over the proportion of lymphocytes.

Foxes G-27 and G-28 (Figs. 7 and 8)

Approximately 2,000 embryonated eggs were given to these foxes and on the twenty-first day after infestation the proportion of basophiles reached

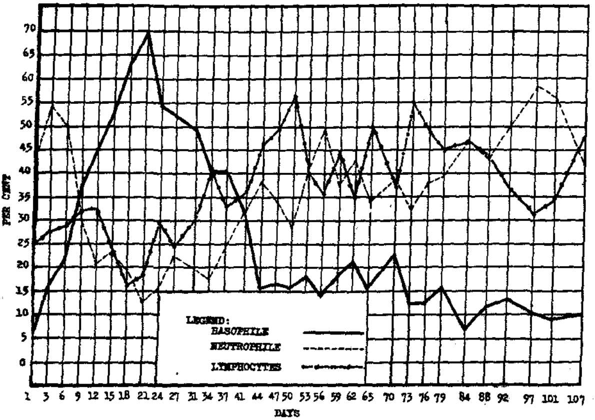

FIG. 7.

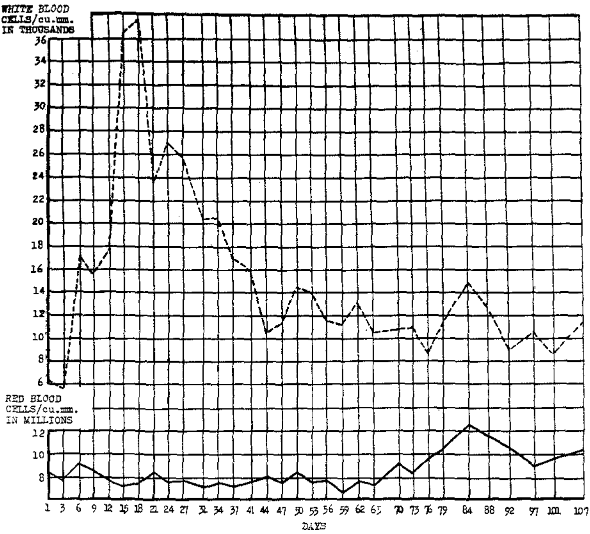

FIG. 8.

69%, decreasing to 15.5% forty-four days later. On the seventieth day there was an increase to 23%, and on the eighty-fourth day the level of 7.0% was reached. As the proportion of basophiles reached the highest peak the proportions of neutrophiles and lymphocytes decreased. After the basophilic low level was reached, the proportion of lymphocytes followed a trend similar to that of the proportion of basophiles and maintained higher levels throughout the experiment than did the proportion of neutrophiles. As the proportion of basophiles decreased, the proportion of neutrophiles increased, and their trends are almost opposite to each other. The trend of the numbers of white blood cells per cu. mm. followed very closely the trend of the basophiles. A peak of 37,000 was reached eighteen days after infestation. There was a decrease to 10,000 forty-four days after infestation. The decreases for both the total white blood cells and the proportion of basophiles occurred the same number of days after infestation.

Foxes G-37 and G-38 (Figs. 9 and 10)

These foxes were given a preliminary dose of approximately 500 embryonated eggs and ten days later were given a similar dose. After the first dose a slight increase from 8.5 to 13% in the proportion of basophiles occurred, and in eleven days, when the second dose was given, the proportion was 3.5%. The proportion of basophiles then increased until twelve days after the second dose when it reached 49.5%. The proportion then decreased to 9.5% thirty

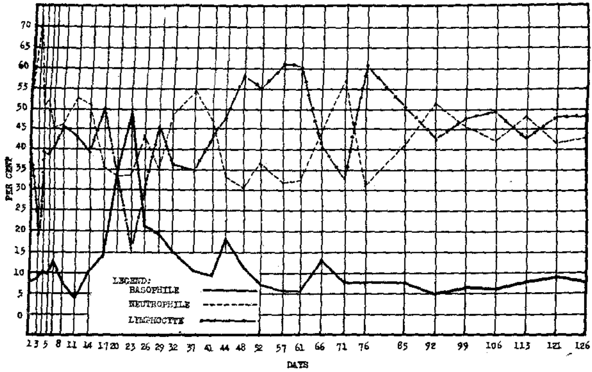

FIG. 9.

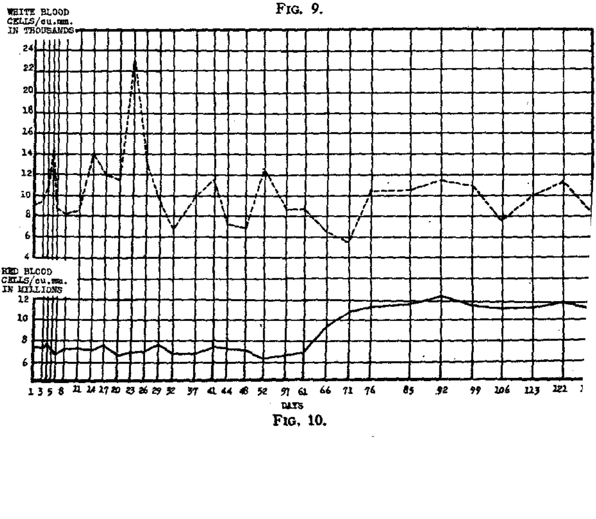

FIG. 10.

days after the second dose. The proportion increased to 18% at thirty-three days, and then showed a downward trend, and at eighty-one days after the second infestation reached 4.5% and remained within normal limits.

As the proportion of basophiles reached the highest peak the proportions of neutrophiles and lymphocytes decreased, but these increased again when the proportion of basophiles began to decline. The proportion of lymphocytes was greater than the proportion of neutrophiles thirty-three days after the second infestation and remained greater until the conclusion of the experiment. The fluctuations of the neutrophiles are opposite to those of the lymphocytes throughout. The total number of white blood cells per cu. mm. reached the peak of 23,000 twenty-three days after infestation, (which was the number of days in which the proportion of basophiles reached the high point), and declined to a low of 7,000 thirty-two days after infestation. Again the trends for both the total numbers of white blood cells and the proportion of basophiles were strikingly similar.

Summary

The proportion of basophiles rises to a marked degree after administration of embryonated *Ascaris* eggs to foxes. In some cases this proportion is as high as 69.5% of the total leucocyte count. The increase in the proportion of basophiles appears to be influenced by the number of eggs given; the largest dose producing the highest, and the smallest dose the lowest, proportion.

There was a marked relation between the proportions of lymphocytes and neutrophiles. These tended to fluctuate in opposite directions. When the proportion of neutrophiles tended to rise the proportion of lymphocytes tended to decline.

The red blood count and haemoglobin remained within normal limits throughout the experiment.

The high proportion of basophiles tends to coincide with the marked increase in the total number of leucocytes. It demonstrates the fact that *Ascaris* infection can cause a marked rise to as high as 69% in the proportion of basophiles per cu. mm. of blood in the fox. The proportions of neutrophiles and lymphocytes are not involved to the same extent. Their actual numbers per cu. mm. of blood may be somewhat increased when the total numbers of white blood cells and proportion of basophiles reach a high peak but their proportions in the blood tend to be lowered. The increase in the total number of white blood cells over the normal appears to be greatly influenced by the increase in the proportion of basophiles; the lymphocytes and neutrophiles influence the increase to a minor extent only.

Acknowledgments

The authors wish to express their appreciation to Dr. Maurice C. Hall and Dr. Emmett W. Price, Bureau of Animal Industry, Zoological Division, Washington, D.C. for reading and commenting upon the manuscript.

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THE MICROBIOLOGY OF BUTTER

I. THE YEAST AND MOLD COUNT OF BUTTER AS A MEASURE OF CREAMERY SANITATION[1]

By F. W. Wood[2] and H. R. Thornton[3]

Abstract

Evidence is presented to show that the yeast and mold count of butter is inadequate as a sole measure of the microbial content of the butter and of creamery sanitation.

It is recommended that for these purposes the bacterial count be used to supplement the yeast and mold count.

Introduction

The plate counts of yeasts and molds in butter are used extensively throughout Canada. Such counts may have a threefold purpose, *viz.*,

i. They may be used to measure the mold content of butter with a view to lowering the incidence of moldy butter. Consideration will be given to this question in a further communication from this department.

ii. They may be used to increase the keeping quality of butter by maintaining its yeast and mold content at a minimum. A number of authors have reported that there is little or no relation between the yeast and mold counts and the keeping quality of butter.

iii. They may be used as a measure of creamery sanitation. The present paper will be confined to a discussion of yeast and mold counts in this role.

Literature

Macy and Richie (1) review the literature on this subject and present a comprehensive bibliography. Reiteration of such readily available information will not be made here. This review serves to emphasize the need for more specific knowledge in this field.

Procedure

The butter samples were removed with sterile spatulas from two corners of packed butter boxes, thus insuring the inclusion of a surface portion, to sterile 4 oz. screw-top jars. After arrival at the laboratory the samples were melted in a water bath at 42-45° C. and were plated as soon as the butter reached a consistency permitting the use of a pipette. In almost all cases not more than 20 min. elapsed between the melting of the butter and the pouring of the plates with the medium. The usual 1 cc. pipettes were used and these were warmed to facilitate complete removal of the butter. The melted butter and the dilutions were shaken 25 times through a distance

[1] *Manuscript received December 4, 1934.*
Contribution from the Department of Dairying, University of Alberta, Edmonton, Canada. The data contained in this paper are taken from a thesis presented by Mr. Wood to the Committee on Graduate Studies, University of Alberta, in partial fulfilment of the requirements for the degree of Master of Science.
[2] *Graduate Student.*
[3] *Professor of Dairying, University of Alberta.*

of approximately 1 ft. The diluent was either physiological salt solution or tap water and losses due to sterilization were made up by the aseptic addition of sterile solution.

The medium used for the plate counts of bacteria was standard nutrient agar. That for the yeast and mold counts was Bacto Dehydrated Malt Agar acidified to pH 3.5 ± 0.1, since this acidity is rapidly becoming standard. pH measurements were made by the electrometric method* using a saturated calomel-potassium chloride half-cell and a quinhydrone electrode in solidified medium at room temperature. Just prior to plating, acidification with a 1:10 aqueous dilution of 85% U.S.P. lactic acid was accomplished. The amount of acid specified by the manufacturers was not in all cases sufficient to lower the pH to 3.5.

Before pouring the medium, the petri dishes containing the inoculums were placed upon a warm plate to melt the butter. This allowed more thorough mixing with the medium. The inoculums did not reach a temperature above 45° C.

The yeast and mold counts were made after incubation of the plates at 25° C. for five days. This procedure was satisfactory except when a large number of molds were present, in which case it was found preferable to count at the end of three days and before the mold colonies lost their identity through merging. The bacterial plates were incubated at 25° C. for four days.

The yeast and mold colonies were counted in transmitted light with the naked eye. The lumi-lens plate counter used for counting bacterial colonies proved unsatisfactory for yeast and mold work. When necessary a hand lens was used and, in the interests of accuracy, a hand tally was employed throughout. All counts are plate counts and are reported on a per cc. basis.

Mycoderms in colony form resemble molds of the *Oidium* type, while in stained preparations the individual cells are more nearly like yeasts. In the absence of any standard for the classification of these micro-organisms for counting purposes they are included with the yeasts in this investigation, except as noted.

Experimental

Air Contamination during Plating

Contamination by molds from the air seems to be frequent enough to have won for these micro-organisms the pseudonym, "Weeds of the Laboratory". A surprisingly large proportion of modern butters are so low in mold content that air contamination of butter surfaces and plates during analytical procedures might be expected to introduce a percentage error of considerable magnitude.

In order to obtain information on the extent of this error 93 control plates were interspersed with the routine plates on 65 days. The majority of these plates remained sterile. The largest number of colonies appearing on any one plate was two and this number occurred on a total of four plates.

*The potentiometric apparatus used in this work was purchased with funds granted by the National Research Council of Canada.

In another study of this problem 25 open plates containing solidified medium were exposed on the desk where plating was proceeding, on 12 days. The period of exposure varied from 20 to 30 min. Seven mold colonies developed which were confined to six plates and the highest number of colonies on any one plate was two. No yeast colonies were recognized in either study.

Because of the location and construction of the laboratory in which this work was conducted, a high incidence of air contamination was expected. These data indicate that, contrary to expectation, the error introduced into yeast and mold counts of butter by air contamination during plating is likely to be so small as to be negligible.

The Growth of Micro-organisms during Plating

It is frequently convenient during routine butter analysis, and necessary during research, to hold the butter in a melted state for considerable time, although this practice is frowned upon in The Preliminary Butter Report (2). Five samples of butter were held at 43° C. for periods up to four hours, plates being poured from these every hour. In no case was any significant change observed in the counts of yeasts, molds or bacteria during this period.

The Relations of the Numbers of Yeasts, Molds and Bacteria in Butter.

The yeast, mold and bacterial counts of 46 samples of exhibition butter from 13 Alberta creameries are presented in Table I. It is apparent that there is no relation between the counts of these three types of organisms

TABLE I

THE PLATE COUNTS OF BACTERIA, MOLDS AND YEASTS IN 46 SAMPLES OF EXHIBITION BUTTER FROM 13 ALBERTA CREAMERIES

Sample No.	Bacteria	Molds	Yeasts	Sample No.	Bacteria	Molds	Yeasts
1	250	0	2	24	7,800	0	4
2	400	0	2	25	8,700	17	0
3	400	1	4	26	8,900	1	1
4	400	6	7	27	10,600	11	8
5	1,400	0	1	28	12,000	3	5
6	1,400	0	0	29	15,800	1	47
7	1,600	0	0	30	18,800	18	9
8	1,800	0	30	31	19,700	1	93
9	2,000	0	12	32	21,700	1	8
10	2,100	28	0	33	22,100	1	8
11	2,100	28	0	34	22,800	5	17
12	3,000	0	0	35	25,000	0	140
13	3,500	0	0	36	29,000	0	2,140
14	3,800	16	21	37	31,800	2	5
15	4,100	8	0	38	38,000	1	19
16	4,600	1	7	39	44,500	10	1,680
17	4,600	1	157	40	55,000	1	1,900
18	4,600	0	5	41	68,600	0	0
19	5,600	1	8	42	75,400	4	26
20	5,700	2	35	43	130,000	0	12,000
21	6,100	0	0	44	136,000	34	28
22	6,200	12	12	45	400,000	0	480
23	7,200	0	16	46	over 1,000,000	0	0

except that most of the high yeast counts occur in butter having high bacterial counts, but it does not follow that a low yeast count is invariably accompanied by a low bacterial count. The same type of relation is seen to exist in the 44 samples of fresh commercial butter from 16 Alberta creameries as listed in Table II. There can be no doubt that the yeast and mold counts constitute a very incomplete measure of the microbial content of many of these butters.

TABLE II

THE PLATE COUNTS OF BACTERIA, MOLDS AND YEASTS IN 44 SAMPLES OF FRESH COMMERCIAL BUTTER FROM 16 ALBERTA CREAMERIES

Sample No.	Bacteria	Molds	Yeasts	Sample No.	Bacteria	Molds	Yeasts
1	650	1	12	23	27,250	0	8
2	900	0	25	24	33,500	50	360
3	2,000	0	45	25	42,000	4	47
4	2,400	0	0	26	44,300	35	8
5	3,600	3	0	27	46,500	2	230
6	5,600	0	0	28	48,500	4	7
7	6,250	0	57	29	50,000	55	250
8	6,500	1	3	30	55,000	30	180
9	9,000	0	4	31	61,000	0	150
10	9,600	1	0	32	79,000	1	131
11	10,600	0	111	33	80,000	4	4
12	10,900	2	2	34	93,500	0	980
13	12,900	1	1	35	95,500	0	270
14	15,000	45	485	36	106,500	2	990
15	15,500	60	1,120	37	107,500	15	4,500
16	15,500	2	3	38	113,000	80	310
17	15,800	0	7	39	145,000	250	2,350
18	17,000	40	350	40	196,000	1	22
19	17,700	0	3	41	600,000	1	210
20	18,200	0	41	42	1,000,000	0	0
21	21,400	10	940	43	1,000,000	0	12
22	25,000	36	15	44	over 1,000,000	4	31

There appear to be at least three possible explanations for the general lack of correlation between numbers of yeasts, molds and bacteria in butter, *viz*:—

i. Their comparative resistance to pasteurization temperatures.

ii. Their comparative growth rates.

iii. Their comparative resistance to the methods employed in the sterilization of creamery equipment.

A thorough study of the flora of cream pasteurized at high temperatures, as practised in Canadian creameries, has not yet been made and will be necessary before it can be said with certainty that pasteurization has no bearing upon the subject under discussion. However, surveys made on Alberta creameries by Dr. Linneboe, Dairy Analyst, Provincial Department of Agriculture, and by the writers, have not shown high yeast and mold counts or particularly high bacterial counts on the pasteurized cream even when high-count butter was encountered. In almost all cases the evidence

has pointed to insanitary equipment. This is well illustrated in Table III which shows the counts of five samples of cream before and after commercial pasteurization. No yeasts or molds were shown to survive the heat treatment and the highest bacterial count after pasteurization was 33,000.

TABLE III

THE PLATE COUNTS OF THE BACTERIA, MOLDS AND YEASTS IN CREAM BEFORE AND AFTER COMMERCIAL PASTEURIZATION

Cream No.	Raw			Pasteurized		
	Bacteria	Molds	Yeasts	Bacteria	Molds	Yeasts
1	46,000,000	300	7,000	2,500	0	0
2	12,000,000	10	6,600	600	0	0
3	500,000,000	300	15,600	33,000	0	0
4	49,000,000	10	640	1,900	0	0
5	—	—	—	16,800	0	0

In a favorable environment the generation times of bacteria are much shorter than those of yeasts and molds. Bacteria have been known to reproduce as quickly as once in 17 min., but this generation rate in a mixed flora in butter seems unlikely. Yeasts reproduce much more slowly than bacteria, requiring, so it is reported, at least three hours in favorable circumstances. The life cycle of the molds is much longer still. Because of the common practice of holding cream for some hours between pasteurization and churning and the probability of the holding temperature being high enough to allow growth in some cases, it is not difficult to visualize the growth differences which may be effected in such cream. The tendency would be for the bacteria to reproduce much more rapidly than the yeasts or the molds, and the result would be a butter high in bacteria and with few or no yeasts or molds. The same condition may be produced when the butter itself is exposed to high storage temperatures.

To study the comparative resistances of bacteria, yeasts and molds to churn "sterilization", a churn was filled approximately one-third full of water at 80-90° C. and revolved from 20-30 min. It was then drained and filled with a definite quantity of cold water of very low bacterial content. The plate counts of 1 cc. of such rinse water in five trials on widely separated days are reported in Table IV. It is to be expected that butter made in this churn from low count cream would vary considerably in its bacterial content and that its yeast and mold count would be misleading as an index of its microbial condition.

TABLE IV

THE PLATE COUNTS OF THE BACTERIA, MOLDS AND YEASTS IN RINSE WATER FROM A "STERILIZED" CHURN

Trial	Bacteria	Molds	Yeasts
1	52,000	0	0
2	9,000	0	0
3	90,000	0	0
4	176,000	0	0
5	440,000	0	0

The Effect of External Butter Workers

Some interesting observations were made upon butter from churns equipped with the external type of butter worker. An opportunity presented itself for the study of butter manufactured in such churns in seven creameries.

TABLE V

THE PLATE COUNTS OF THE BACTERIA, MYCODERMS AND YEASTS IN 83 SAMPLES OF BUTTER FROM CREAMERY *A*, EQUIPPED WITH A CHURN HAVING AN EXTERNAL BUTTER WORKER

Sample No.	Bacteria	Mycoderms	Yeasts	Sample No.	Bacteria	Mycoderms	Yeasts
1	1,000	0	30	43	18,000	0	290
2	1,000	0	300	44	19,000	1	25
3	1,000	0	230	45	19,000	4	39
4	2,000	0	16	46	19,000	10	230
5	2,000	0	10	47	20,000	0	140
6	3,000	1	23	48	21,000	1	52
7	3,000	2	23	49	23,000	0	190
8	3,000	5	17	50	24,000	0	650
9	3,000	9	14	51	24,000	40	450
10	4,000	1	63	52	24,000	30	130
11	4,000	20	60	53	26,000	1	69
12	4,000	0	620	54	27,000	0	110
13	4,000	40	320	55	28,000	19	26
14	4,000	11	34	56	29,000	10	6
15	5,000	0	1	57	30,000	9	29
16	5,000	12	80	58	30,000	0	22
17	5,000	10	20	59	30,000	11	95
18	6,000	36	21	60	33,000	10	10
19	6,000	7	9	61	37,000	10	91
20	7,000	30	5	62	38,000	0	160
21	9,000	3	20	63	39,000	0	150
22	9,000	29	27	64	43,000	1	11
23	9,000	0	40	65	44,000	1	13
24	9,000	0	230	66	46,000	1	13
25	10,000	52	44	67	50,000	0	10
26	10,000	50	710	68	54,000	0	81
27	11,000	30	460	69	56,000	0	140
28	11,000	20	440	70	56,000	0	100
29	11,000	21	129	71	61,000	50	120
30	11,000	0	188	72	65,000	26	1
31	11,000	0	380	73	95,000	4	180
32	13,000	20	610	74	100,000	20	20
33	14,000	40	290	75	132,000	10	350
34	15,000	3	430	76	136,000	0	110
35	15,000	0	38	77	160,000	2	0
36	16,000	28	25	78	160,000	0	2
37	16,000	20	20	79	170,000	4	32
38	16,000	0	130	80	188,000	0	23
39	17,000	36	70	81	286,000	30	10
40	17,000	0	8	82	325,000	10	30
41	18,000	0	47	83	428,000	10	550
42	18,000	0	26				

This butter was characterized by a rather regular, high, yeast and mycoderm content. Table V shows the bacterial, yeast and mycoderm counts of 83 samples of butter from a representative churn of this type, the counts being arranged in ascending order of the bacterial counts. It is seen that there is

no tendency for the mycoderm or yeast counts to increase with an increase in the bacterial counts. Similar counts of butter from consecutive churnings from two other creameries equipped with external butter workers are given in Table VI. Churning number 5 of Creamery *C* immediately followed the dismantling and thorough cleaning of the butter worker.

The yeast and bacterial counts of 70 samples of butter manufactured in 11 creameries known to be equipped only with churns having internal workers are set forth in Table VII. The mycoderm count of each sample was either zero or was so low that it was not considered separately from the yeast count. If the churns which we studied are representative then it seems probable that the external type of butter worker may contribute large numbers of yeasts and mycoderms to butter. One such worker was dissembled and decomposing material taken from one of the joints gave the following analysis per gm.: bacteria 1,000,000,000; yeasts 10,000,000; mycoderms 1,300,000. This material does not show the same relation between the counts of the different kinds of micro-organisms as is shown by the manufactured butter. Two possible explanations are:

1. The inadequacies of the plate method of analysis.

2. An equilibrium, differing from that above, which may exist at the very surface or edge of the material *in situ* from which the contamination of the butter takes place.

Discussion and Recommendation

It is common—almost to the point of being universal—practice in Canadian creamery butter manufacture to pasteurize cream at not less than 170° F. for 10 min. and starters are not used for cream ripening after pasteurization. The bacterial content of butter so made, therefore, has a different significance than when low-temperature pasteurization and starters are used. In these circumstances a very high bacterial content in the finished butter denotes one, or a combination, of the following faults:—

i. Large numbers of thermoduric bacteria in the raw cream. This seems unlikely to happen but is included here in the absence of more extensive proof to the contrary than is offered in this paper. In any case this would probably constitute a serious defect in butter.

ii. Improper pasteurization.

iii. Growth after pasteurization either in the cream or in the butter.

iv. Contamination subsequent to pasteurization.

It is believed that the evidence presented in this paper shows conclusively that the yeast and mold count of butter is an incomplete measure of its microbial content and is frequently misleading when so used. Therefore, it is recommended that, if the maximum reduction of the number of micro-organisms in Canadian butter is to be effected, the yeast and mold count be supplemented with the bacterial count.

TABLE VI

THE PLATE COUNTS OF BACTERIA, MYCODERMS AND YEASTS IN CONSECUTIVE CHURNINGS FROM CREAMERIES *B* AND *C*, EACH EQUIPPED WITH CHURNS HAVING EXTERNAL BUTTER WORKERS

Churning No.	Creamery *B*			Creamery *C*		
	Bacteria	Mycoderms	Yeasts	Bacteria	Mycoderms	Yeasts
1	53,000	60	610	8,000	0	470
2	18,000	20	650	8,000	0	160
3	27,000	30	630	7,000	30	110
4	39,000	90	750	33,000	0	310
5	53,000	20	900	1,000	0	10
6	31,000	50	320	30,000	0	0
7	40,000	40	580	12,000	170	700
8	27,000	20	430	1,000	140	60
9	54,000	20	910	1,000	310	70
10	13,000	30	270	1,000	60	100
11	26,000	70	340	1,000	360	30
12	116,000	270	1,110	5,000	20	90
13	38,000	70	430			
14	76,000	110	1,390			

TABLE VII

THE PLATE COUNTS OF BACTERIA AND YEASTS IN BUTTER FROM 11 ALBERTA CREAMERIES EQUIPPED WITH CHURNS HAVING INTERNAL BUTTER WORKERS

Sample No.	Bacteria	Yeasts	Sample No.	Bacteria	Yeasts
1	100	0	36	8,000	0
2	200	2	37	8,000	1
3	500	0	38	8,000	0
4	500	9	39	8,000	0
5	600	0	40	8,000	50
6	1,000	0	41	9,000	230
7	1,000	0	42	9,000	2
8	1,000	0	43	10,000	240
9	1,000	0	44	11,000	79
10	1,200	1	45	13,000	0
11	1,500	4	46	13,000	85
12	1,500	0	47	13,000	0
13	1,700	0	48	14,000	4
14	1,700	0	49	14,000	0
15	2,000	1	50	15,000	20
16	2,000	0	51	17,000	5
17	2,600	0	52	18,000	2
18	3,000	2	53	18,000	0
19	3,000	3	54	19,000	0
20	4,000	11	55	21,000	0
21	4,000	0	56	23,000	21
22	4,000	0	57	23,000	11
23	4,000	10	58	24,000	2
24	4,000	3	59	26,000	12
25	4,000	0	60	30,000	59
26	5,000	0	61	36,000	0
27	5,000	0	62	42,000	1,370
28	5,000	0	63	43,000	104
29	5,000	0	64	135,000	20
30	5,000	0	65	160,000	740
31	6,000	2	66	335,000	56
32	6,000	0	67	360,000	4
33	7,000	0	68	364,000	213
34	7,000	0	69	640,000	28
35	8,000	32	70	over 6,000,000	570

Summary

1. Air contamination of plates and butter surfaces with yeasts and molds during plating is likely to be small.

2. No significant changes were observed in the bacterial, yeast and mold counts of five samples of butter held in the melted state for four hours.

3. The only relation observed between the different kinds of micro-organisms in butter was that high yeast counts tended to occur in butter of high bacterial content.

4. The possibility of the differing growth rates of bacteria, yeasts and molds and their resistance to equipment "sterilizing" procedures being responsible for the general lack of relation between the counts of these organisms in butter is discussed.

5. Butter manufactured in seven churns equipped with external butter workers was characterized by a high yeast and mycoderm count.

6. In view of the manufacturing practices common in creameries in Canada a very high bacterial content in Canadian creamery butter is evidence of a fault in the production or manufacture of the butter.

7. The yeast and mold count of butter is an incomplete measure of its microbial content and consequently of creamery sanitation.

8. It is recommended that consideration be given to supplementing the yeast and mold count of Canadian creamery butter with the bacterial count.

References

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THE MICROBIOLOGY OF BUTTER

II. THE GROWTH OF MOLDS IN AND UPON BUTTER[1]

By. H. R. Thornton[2] and F. W. Wood[3]

Abstract

The improbability of the initiation of mold growth in the incorporated moisture of salted and unsalted butter and in the free moisture of salted butter is discussed. An attempt is made to relate the initiation of mold growth to condensation moisture on the various surfaces of butter. Experiments showing the comparative effect on the initiation of mold growth of four methods of parchment treatment are described. *Cladosporium* sp. was isolated from a butter showing the interior type of molding.

Introduction

Although the creamery butter of Alberta has been subjected to a yeast and mold control program of ten years' duration, moldy butter continues to be a source of economic loss. Its sporadic and puzzling recurrence on prints and solids suggests that the control measures have failed to reach the vital spot of the trouble. Dependence has been placed almost solely on the lowering of the mold content of the butter although this is but one of many factors which may influence the growth of molds.

Our knowledge of the physical and chemical structure of butter is yet too incomplete to permit prediction, or recognition with certainty, of all the conditions affecting the growth of molds in or upon butter. It is possible, even probable, that adequate control of the molding of butter will be deferred until more information relating to the chemistry of butter is available. In the meantime there is nothing to be lost in maintaining a viewpoint which takes cognizance of the few available facts regarding the chemical nature of butter on the one hand and the growth requirements of molds on the other. The present discussion is an attempt to correlate some hitherto apparently contradictory observations and to make some small contribution to our knowledge of the subject.

Procedure

The general plating procedure described in the first paper of this series was followed in the present investigation.

One-pound prints of commercial creamery butter low in mold content were each divided into two approximately equal parts. One part was inoculated with mold spores while the other was left uninoculated. Each part was then subdivided into portions of approximately one-tenth pound each. These portions were wrapped as uniformly as possible with parchment treated as described later. Unless otherwise stated, reference will be made only to salted butter.

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Contribution from the Department of Dairying, University of Alberta, Edmonton, Canada. The data contained in this paper are taken from a thesis presented by Mr. Wood to the Committee on Graduate Studies, University of Alberta, in partial fulfilment of the requirements for the degree of Master of Science.
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Two cultures of *Penicillium*, isolated from moldy butter and parchment, were the source of the inoculum. Slants of these strains, sufficiently aged to contain an abundance of spores, were employed and the inoculum was mixed as thoroughly as possible with the butter to effect even distribution of the spores. Owing to the fact that the action of specific molds was not being studied no precautions were taken to maintain aseptic conditions during the inoculation and wrapping of the butter. It is probable that many of these samples became inoculated with extraneous molds during the manipulations of preparation and observation. Therefore, while these results can be interpreted with certainty only for the two cultures of *Penicillium* used, it is possible that they are representative for many species. After the butter was wrapped mold spores were sprinkled on the outer surfaces of the parchment in the case of butters 1, 4 and 5 (Table I).

The four methods of treating the parchment prior to wrapping were as follows:—

i. Sterile parchment—dampened and autoclaved for 1 hr. at 15 lb. pressure.

ii. Hot brine —soaked for 1 hr. at 97° C. in brine which was saturated with sodium chloride at room temperature.

iii. Cold brine —soaked in saturated sodium chloride solution for several hours at room temperature.

iv. Dry parchment —no treatment.

Incubating the samples together in one large humidifier proved unsatisfactory because of the collection of condensed moisture and the transference of this moisture with salt from one sample to another. Each sample was, therefore, placed in a sterile screw-top ointment jar in the bottom of which was 2 cc. of sterile water. These jars were then incubated at 12-15° C. in a chamber with a moisture saturated atmosphere.

Types of Molding

Two types of mold growth on butter are frequently confused both in the industry and in the literature. In the first type, mold growth occurs on the surface of the butter and—another instance of the same thing—in the cracks, crevices and creases of poorly packed butter. This type will be termed surface molding in the present discussion. It occurs very frequently on commercial butter but its presence does not always mean large monetary loss since the outer layers of butter can be removed leaving the freshly cut surface free from mold growth.

The second type of molding occurs as smudgy, dark areas or patches throughout the interior of the butter mass. This type is encountered comparatively rarely but its occurrence usually results in almost complete loss of the butter since it cannot be readily removed and the butter is unmarketable. In the present investigation it will be referred to as interior molding, and discussion relating to it will be confined to the section entitled "Interior Molding".

Salt Relations

Salt Concentration in the Butter

After making an extensive review of the literature of this subject Macy (4) concludes that "the influence of salt varies depending upon the species and the substrate. Altho unsalted butter is most commonly affected, mold often appears on salted butter. This occurrence demands explanation."

In the present investigation the ease with which mold growth can be induced on the surface of unsalted butter was noted. At the same time no sample of salted butter was encountered which did not become moldy when stored under favorable conditions. The maximum salt concentration of the butters used in these experiments was 2.5%.

While there is a degree of uniformity in the results of different investigators, the divergencies which appear are explainable, in part at least, by known factors. It is not to be expected that mold growth is initiated in the fat itself. Mold growth will, therefore, be influenced not by the salt concentration of the butter but by the salt concentration of the butter moisture. The salt concentration of the butter moisture is governed not alone by the percentage of salt and moisture in the butter but by the distribution of the salt and moisture as well. Microscopic examination of butter usually reveals undissolved salt crystals—proof that the salt is not homogeneously distributed. The travel of moisture in salted butter is evidence of a tendency for the salt to become homogeneously distributed throughout the moisture. But, owing to the slow diffusion of moisture through butter, an entirely homogeneous distribution of the salt is very improbable. It is apparent that the percentage of salt in the butter is not a satisfactory measure of the inhibitory effect of salt even when consideration is given to the salt tolerances of different molds. But it is highly probable that the salt concentration of the butter moisture of commercial salted butters is sufficiently high to act as an important inhibitory agent in view of the other unfavorable conditions of butter storage. This probability receives support in the observation that unsalted butter becomes moldy with ease and in the fact that the growth of mold on different salted butters of the same salt and moisture concentration is not more erratic than the growth of mold upon the butter in different boxes of the same churning. The fact that all the butters observed in this work became moldy when conditions other than salt concentration were optimum points to something other than salt as the controlling factor in the case of salted butters.

Salt on the Butter Wrappers

Salt has proved to be an effective agent in the prevention of mold growth on the parchment liners and wrappers used in the packaging of butter. A number of investigators recommend boiling the parchment in saturated salt solution. Macy and Pulkrabek (5) present an excellent review of the literature on this subject.

In recent years some creameries have resorted to the use of dry parchment as received from the manufacturer for the wrapping of print butter. It has been reported to us that the adoption of this practice has been attended

with some success in reducing the incidence of moldy butter. It is claimed that this method of controlling mold growth on butter has been used with success in New Zealand.

Data are presented in Table I to show the effect of parchment treatment upon subsequent mold growth both on the butter and on the parchment on incubation for periods varying up to 21 weeks in a moisture-saturated atmosphere. It is seen that a wet sterile wrapper gives the least protection against moldiness, while dry wrappers were fairly effective for three weeks but not for eight weeks. Wrappers soaked in saturated salt solution, either hot or cold, gave the maximum protection.

Oxygen Relations

In reviewing the effect of oxygen upon mold growth on butter, Macy (4) concludes that "The notion that molds are strict aerobes seems to be faulty, but it is evident that they do require some oxygen for their normal development, altho excessive quantities are inhibitory." He was able to obtain meagre growth on butter in partial pressures of oxygen and no growth in the absence of oxygen. In the present investigation initiation of growth of any mold studied was not observed on acidified malt agar in the complete absence of oxygen in Spray anaerobic culture dishes. The fact that mold growth is observed infrequently in butter at any place other than the surface seems to justify the conclusion that mold growth in or upon butter implies the presence of considerable quantities of oxygen. Therefore, any theory in explanation of the molding of butter will have to presuppose the presence of oxygen.

The modern conception of organic complexes in solution is that they respire, consuming oxygen. The moisture of butter contains an organic complex in solution. Therefore—by analogy—it consumes oxygen. When this moisture is *in situ* in the butter the diffusion of oxygen must necessarily be very slow. Because of this it is highly probable that an anaerobic state is rapidly reached in this moisture in salted and unsalted butter after manufacture. The theory that mold growth is initiated in the incorporated butter moisture then becomes untenable.

When the butter moisture "leaks" onto the surface it is probable that the diffusion of oxygen from the atmosphere is more rapid than the consumption of oxygen by the butter moisture, and aerobic conditions result. This assumption is justified by the behavior of other solutions of organic complexes. The salt in the moisture of salted butter then tends to inhibit the growth of molds in the expressed moisture.

Shutt (7) claims that "*Alternaria*, *Spicaria* and *Oospora* types can exist in the butter and spread rapidly when the mycelium reaches the surface, where more oxygen is available. Perfect union between butter and wrapper seems to be no hindrance to the growth of these molds." Not only do these two statements seem to conflict but the author presents no data in support of his contentions and does not describe the technique by which he was able to make

TABLE I

THE OCCURRENCE OF MOLD ON THE PARCHMENT WRAPPERS AND ON THE SURFACE OF INOCULATED AND UNINOCULATED COMMERCIAL BUTTER AFTER INCUBATION AT 12-15° C. FOR VARYING PERIODS IN A MOISTURE SATURATED ATMOSPHERE

Butter No.	H_2O %	Salt %	Inoculation	Mold count	Location of molding	Sterile parchment			Hot brine			Cold brine			Dry parchment		
						3 weeks	13 weeks	21 weeks	3 weeks	13 weeks	21 weeks	3 weeks	13 weeks	21 weeks	3 weeks	13 weeks	21 weeks
1	14.7	2.5	Uninoculated	2	Parchment Butter	+ −	+ +	+ +	− −	− −	+(1) −	− −	− +	+(1) +	− −	− −	+ −
	14.2	2.2	Inoculated	100,000	Parchment Butter	− −	+ −	+ −	− −	− −	− −	− −	− −	− −	− −	+ −	+ +
2	13.7	1.7	Uninoculated	0	Parchment Butter	+ −	+ +	+ +	− −	− +	+ +	− −	− +	− +	− −	+ +	+ +
	13.2	1.6	Inoculated	100,000	Parchment Butter	− −	+ −	+ −	− −	− −	− −	− −	− −	− −	− −	+ −	+ −
3	15.0	1.2	Uninoculated	5	Parchment Butter	+ −	+ +	+ +	− −	− +	+ +	− −	− +	+ +	− −	+ +	+ +
	14.7	1.1	Inoculated	60,000	Parchment Butter	− −	+ −	+ +	− −	− −	+ +	− −	− −	+ +	− −	+ −	+ +
						8 weeks	16 weeks		8 weeks	16 weeks		8 weeks	16 weeks		8 weeks	16 weeks	
4	13.5	1.2	Uninoculated	10	Parchment Butter	− −	+ +		− +	+ +		− −	+ +		− −	+ +	
	13.6	1.2	Inoculated	380,000	Parchment Butter	+ −	+ +		− −	− −		− −	− −		− −	(2) (2)	
5	14.1	1.4	Uninoculated	10	Parchment Butter	+ +	+ +		− −	− +		− −	− +		+ +	+ +	
	13.1	1.4	Inoculated	780,000	Parchment Butter	− +	+ +		− −	− −		− −	− −		− −	+ +	

NOTE:—(+) *Growth.* (−) *No growth.* (*1*) *Growth very slight.* (*2*) *Results not recorded.*

such observations. Many workers do not differentiate between the growth of molds and the initiation of growth. The latter is the important consideration in the question under discussion. No theory of the structure of butter yet propounded suggests that the conditions immediately below the surface of butter differ from those in the farther interior. If the quoted statements are true, we are at a complete loss to explain the infrequent occurrence of the interior type of molding.

Some authors have referred to molds growing through parchment. During the course of the present study the piercing of parchment by molds has not been observed and proof of this ability was not forthcoming with any of a number of techniques devised for the purpose.

Moisture Relations

The great lack of uniformity in the occurrence of moldiness on different samples of butter of the same brine concentration and even on different boxes of the same churning indicates that the key to control lies not in the butter moisture or salt concentration. Without exception, investigators who discuss this subject report the necessity for storage of the butter in atmospheres of high relative humidity in order to induce macroscopic evidence of mold growth (4). Shutt (7) was able to germinate and grow on the outer surfaces of butter wrappers the varieties of molds with which he worked when the relative humidity of the storage rooms was 100%, but was unable to do so when the relative humidity dropped to 90%. Tomkins (9), while studying the relation of mold growth on different surfaces to the relative humidity of the atmosphere, found that the spores of *Alternaria citris* germinated and the mycelium grew to a length of 7.7 μ in an atmosphere with a relative humidity of 100% and only 0.3 μ when the relative humidity was 90.8%. Skovholt and Bailey (8) failed to obtain macroscopic evidence of mold growth on bread crumbs held in atmospheres of less than 90% relative humidity.

In the present investigation no sample of butter became moldy on incubation for 60 days at ice-box or room temperature and humidity. Every sample quickly became moldy when removed to a moist chamber at the same temperatures. It is apparent that, apart from temperature, the humidity of the storage atmosphere is the greatest single factor influencing the molding of butter, and the evidence to date does not favor the theory that mold growth is initiated in the butter moisture, either free or incorporated.

Atmospheric humidities are related to the size of moisture condensation films on surfaces coming into contact with the atmosphere. The condensation moisture on the surface of butter is likely to exist in the form of droplets rather than as continuous films due to surface tension effects. The work of Collins and Hammer (1) on the migration of bacteria through butter lends support to this theory. An exception would occur where the parchment forms a capillary space with the surface of the butter, at which point the moisture would form as a film. In our experimental work the collection of moisture

in the folds and creases of the parchment during storage at high humidities has at times been so excessive as to cause the moisture to run off the parchment, carrying with it sufficient salt to change the results of the experiments. Many samples of butter in commercial storage were observed to be moldy at those places where moisture would be expected to collect in large amounts if the condensation moisture had been excessive. Condensation moisture would contain no salt while it would quickly dissolve nutrient material from the butter and the parchment. Mold could, therefore, grow in this solution. If the condensation moisture came into contact with the salted butter moisture, the salt concentration would be lowered and its inhibitory effect lessened. Support is given to this theory in the investigations of Vernon (10) who found that free moisture on the surface of butter was superior to pure water as a pabulum for molds.

If the foregoing is representative of the conditions existing on the surface of butter, then control measures will have to include methods of preventing, as far as possible, the formation of condensation moisture. Observations both in the laboratory and in the industry are to the effect that the storage of butter in atmospheres of low humidity tends to decrease the precipitation of moisture on the butter surfaces. The more carefully the butter is packed the less will be the tendency to leave microscopic or capillary spaces between the butter and the parchment and unnecessary creases or folds in the parchment.

As has been pointed out by previous authors, there is another source of salt-free moisture in the manufacture of butter. This is rinse water, water from wet equipment, etc. which falls on the finished surface of the butter or is carried there by wet parchment. Like the condensation moisture this water is likely to be at oxygen pressure equilibrium with the atmosphere. It will dissolve nutrient material from the butter and parchment—thus permitting mold growth—and will dilute the free butter brine which may be expressed onto the surface of the butter. The control measure which immediately suggests itself is to keep this type of moisture away from butter.

Temperature Relations

The influence of temperature on the growth of molds on butter is too well known to justify enlargement here. Suffice it to emphasize that the temperatures of butter storages in this province cannot always solely be depended upon to inhibit mold growth but that the incidence of this butter defect is lessened as temperatures are lowered.

There is, however, another relation dependent upon temperature which deserves consideration. The air which is incorporated into butter is the air which is in the churn during manufacture. This air is incorporated at churning temperature and is likely to have a relative humidity approaching 100%. The air which may be entrapped contiguous to the surface during the packing and wrapping of the butter will be that of the churn room and will likely be high in temperature and humidity. When the butter is stored,

the temperature of this air will fall and moisture will be precipitated from it on the butter surfaces, even on those of the incorporated air pockets. When the butter is again removed to higher temperatures, more moisture will be condensed from the warmer atmosphere on the cold surface of the butter and particularly on the parchment. Vernon (10) reports an increased incidence of moldy New Zealand butter in London during warm weather and relates the phenomenon to a greater condensation of atmospheric moisture on the cold surfaces of butter when it is removed from storage.

Contamination Relations

A number of Alberta buttermakers have reported to us their experience that the molding of commercial butters seems to bear no relation to the mold count of the butter. A few such butters were investigated by us and the results substantiate the opinion of the practical men. Our attention has been called to the fact that the incidence of moldy butter has decreased with the decrease in the mold content of the butter in one of the provinces of Canada. This, however, does not constitute proof that the two phenomena are directly related in the absence of information regarding the other variables.

Combs and Eckles (2) were unable to find any relation between the mold count and the subsequent molding of the butter. Shutt (7) on the other hand states that "molds can develop on the butter under the wrapper when the initial mold spore contamination of the butter stored is very high." He presents no data in support of this statement.

In the present investigation no sample of butter was encountered which became moldy when the humidity of the storage atmosphere was unfavorable. No sample failed to become moldy when the humidity of the storage atmosphere was favorable. Scrutiny of Table I reveals that there is no relation between the mold count and the subsequent development of moldiness either on the inoculated or uninoculated butters there reported. Our observations, therefore, confirm those of Combs and Eckles.

These results appear reasonable when consideration is given to all the factors affecting the growth of molds upon butter. Apart from temperature, the humidity of the atmosphere surrounding the butter has more influence upon the development of moldiness than any other single factor and, probably, than all other factors combined. In the commercial handling of butter this is likely to vary more than any other condition, the variations being sufficient to account for the erratic appearance of molding in different boxes of the same churning.

Many of the incorporated mold spores and mycelial portions are likely to be imbedded in the fat of the butter and cannot, therefore, initiate growth. There is no evidence that incorporated spores can initiate growth of the surface type in salted butter. Common observation suggests that, if this occurs at all, it does so infrequently and cannot constitute the major cause of this type of molding. It is highly probable that few or no samples of commercial butter are finished without mold spores or mycelium dropping on their

surfaces. It is possible that these unincorporated mold spores and mycelial portions constitute the major source of trouble.

The results here cited should not be interpreted as meaning that the authors consider the mold count useless in the control of moldy butter. They do not constitute proof that there is no relation between the mold content of butter and the subsequent development of mold. But they can be taken as evidence that, if any relation exists, the methods of measurement at present available are not sufficiently exact to detect it. Moreover, an indirect relation between the mold count and the incidence of moldiness probably does exist. Any campaign which has for its purpose the lowering of the mold count of butter will undoubtedly eliminate many sources of external contamination.

Interior Molding

The fact that the interior type of molding is of comparatively rare occurrence while surface molding is easily induced upon commercial butter immediately focuses attention upon the peculiar chemical and physical structure of butter. It has already been pointed out that the theory that mold growth is initiated in the incorporated butter moisture is untenable. Butter is known to contain appreciable quantities of air. Pickerill and Guthrie (6) found from 0.5 to 14.9% of the butter volume to be air, with an average of 4%. Although the nature of the distribution of this air is not well known, it is apparent that a considerable proportion of the microscopic droplets in butter—usually called moisture droplets—are in reality air globules. King (3) points out the difficulty of recognizing these air globules with certainty in the microscopic examination of butter.

The incorporated air in butter comes from the air in the churn. This air is high in humidity and on the temperature being lowered during storage condensation moisture will be deposited in minute droplets at the air-oil interface. The microscopic picture of many of the droplets in butter is such as to suggest that this has taken place. The conditions at the air-oil interface within the butter become not entirely unlike those at the outer surface of the butter and an environment favorable for mold growth is provided.

An opportunity presented itself for a microscopic examination of a butter displaying this type of mold growth. When material from the dark smudgy areas in the butter was viewed under the microscope it was observed that all growth originated in droplets and it was assumed that these were air droplets. In many of these droplets the exact point of growth initiation was recognizable and was invariably at the air-oil interface. The mycelium then grew in the droplet or globule, frequently filling this with a coil of mycelial growth which was sometimes unable to pierce the butter. At other times the mycelial growth penetrated the butter for considerable distances, often through other droplets. These observations emphasize the difference between the point of initiation of growth and the direction of subsequent mycelial growth.

A mold was isolated from the moldy patches and was identified by three independent workers as *Cladosporium* sp. Morphologically it was indis-

tinguishable from the mold observed *in situ* in the butter. The mold count of this butter was low and no species of *Cladosporium* was observed on the plates. An explanation was provided for this in that the mold which was isolated would not grow into macroscopic colonies in the five-day incubation period. It is interesting to note that other workers have reported *Cladosporium* sp. as the cause of this type of molding (10).

Summary

1. Two types of butter molding are at present recognized, *viz.*,— surface molding, which occurs frequently and interior molding, which occurs infrequently.

2. Emphasis is placed upon sharp differentiation between the point of initiation of mold growth and the direction of subsequent mycelial growth.

3. The evidence to date is interpreted as indicating that mold growth is not initiated in the butter moisture, either incorporated or expressed, of salted butter because,

(*a*) Salt is an important inhibitory agent, especially in the presence of other unfavorable conditions inherent in commercial butter storage.

(*b*) Incorporated butter moisture is likely to become anaerobic within a short time after manufacture.

4. No sample of butter was encountered which became moldy at ice-box temperature when the humidity of the surrounding atmosphere was low. No sample failed to become moldy at the same temperature when the humidity of the surrounding atmosphere was high.

5. Condensation moisture is suggested as the most probable major factor, apart from temperature, controlling the molding of butter. Butter should be handled and stored, therefore, in such a manner that the minimum of condensation moisture will be formed.

6. No relation was found between the mold count of butter and the subsequent development of moldiness.

7. Of four methods of treatment, wet sterilization of the parchment was the least satisfactory, while parchment soaked in hot or cold saturated brine gave the maximum protection against subsequent mold growth.

8. *Cladosporium* sp. was isolated from a moldy patch in butter showing interior molding. The point of initiation of growth was observed and is described.

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WEATHER AND WHEAT YIELD IN WESTERN CANADA

I. INFLUENCE OF RAINFALL AND TEMPERATURE DURING THE GROWING SEASON ON PLOT YIELDS[1]

By J. W. Hopkins[2]

Abstract

A statistical study of plot yields recorded at a number of agricultural experiment stations in central and southern Saskatchewan and Alberta has demonstrated a significant correlation between yield and the amount and distribution of seasonal rainfall. On the whole, above-average rainfall is associated with higher yield, but the result of a given increment of rain at different times is partly dependent on soil conditions. On fertile soil, rainfall prior to harvesting results in a reduction of yield, probably owing to lodging. The maximum influence of precipitation upon yield appears to be exerted during the month of June. The average summer rainfall sequence is very similar in each of the above four districts. There is a moderate degree of correlation between the amounts of rain recorded in different districts during the same season, but the simultaneous occurrence of extremely wet or dry seasons over the whole area seems infrequent.

Temperature conditions during the growing season seem to be secondary to rainfall in influencing yield. Above-average temperatures are beneficial at the time of sowing, detrimental during mid-summer and again beneficial prior to ripening, but as in the case of rainfall, the effect produced is influenced by soil conditions. No consistent relation is evident between either rainfall or temperature and the relative yield of early and late maturing varieties.

It is apparent that the yields secured are influenced by factors other than those considered, and the precipitation during the autumn, winter and spring months prior to sowing is being studied in this connection.

I. Introduction

It is generally believed that the large fluctuations in the annual yield of wheat and other Canadian field crops are to a considerable extent attributable to variations in the weather conditions prevailing in different seasons. Apart from certain broad generalizations, however, little can be said to be definitely known at present concerning the actual quantitative relations involved and the extent to which these may be modified by soil conditions or cultural practices.

Estimation of these effects is complicated by the fact that, during the growing season at least, equal increments of rainfall, heat, etc., occurring at different times cannot be assumed to exert a similar influence upon yield; for in addition to meteorological considerations (13), the requirements of the plant do not remain constant throughout the growth cycle (*e.g.*, 6, 14, p. 67). Several agricultural meteorologists have therefore been led to postulate the existence of "critical periods" in crop development. At these times (which may be of relatively short duration) the plant is supposed to be particularly sensitive to an excess or defect of some meteorological element, of which fluctuations in the then available amount exercise a major influence upon yield (1, 2, 3, 4, 7, 12, 17).

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Contribution from the National Research Laboratories, Ottawa. Abstracted from a thesis approved for the degree of Ph.D. in the Faculty of Science, University of London. Published as Paper No. 73 of the Associate Committee on Grain Research.

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In this connection it was observed during the course of wheat varietal tests at the University of Alberta that in some years marked differences in the relative order of yield of early and late maturing varieties occurred, and it appeared possible that these fluctuations in relative yield were partly attributable to some differential effect of weather conditions upon the varieties. Knowledge of any such differential effects upon varietal yields would be of considerable practical importance.

The present exploratory investigation was, therefore, undertaken with the object of studying the relation between rainfall and temperature conditions during the growing season and the yield of a specified variety of wheat, and also the relation between these weather factors and the relative yield of early and late maturing varieties. The method employed is the statistical one, in which numerical measures of the weather conditions are correlated with the associated crop yields with the object of deducing the quantitative relations between them.

2. Observational Data

Owing to the nature of the relations which it is sought to investigate, a relatively large number of statistical parameters must be calculated in order to specify them. This in turn necessitates an extensive series of observations, if reliable results are to be obtained. Fisher, for example, investigating the influence of rainfall upon the yield of wheat at Rothamsted (10), utilized the records of 60 harvests from Broadbalk field.

In Western Canada continuous crop records of this extent are naturally nowhere available. At the various Dominion Experimental Stations throughout the prairie provinces, however, and also at the agricultural colleges of the provincial universities, plot yields of the more commonly grown wheat varieties have been recorded for a number of years. An attempt has, therefore, been made to combine the experience of a number of these stations in order to arrive at an estimate of the average relation in the area which they represent. This, being based on a more extensive body of observations than is available at any single station, may be expected to be correspondingly more reliable, though it must be recognized that the relations thus deduced are probably not exactly representative of the situation at any individual station.

In Tables I and II the annual yields in the variety test experiments referred to are recorded, the values in each case representing the average performance of replicate plots. These were obtained from the annual reports of the directors of the various Dominion Experimental Stations and from records supplied by the Department of Field Husbandry, University of Saskatchewan, and Department of Field Crops, University of Alberta. Various members of the Experimental Farms Branch also assisted by supplying information not available in published form.

These trials are commonly carried out on well prepared summerfallow land, the fertility of which is maintained by the rotation of leguminous or other crops. The investigation has, therefore, been extended to a further series of crop returns, obtained under less favorable conditions. This comprises

TABLE I

YIELD, IN BUSHELS PER ACRE, OF MARQUIS WHEAT GROWN IN VARIETY TEST PLOTS

Year	Edmonton[1]	Lacombe	Lethbridge	Indian Head	Scott	Rosthern	Saskatoon[2]
1931	54.6	47.2	17.0	16.1	29.3	17.6	19.6
1930	33.0	46.7	20.8	39.0	42.6	29.8	42.6
1929	29.0	43.7	27.8	31.0	18.7	42.4	26.1
1928	46.4	36.9	42.5	45.4	36.2	30.7	51.0
1927	59.9	40.5	26.7	41.1	48.8	31.5	36.1
1926	35.6	46.4	38.1	49.2	24.3	18.5	38.8
1925	44.6	55.9	25.1	45.8	33.7	36.7	36.6
1922	22.6	24.3	26.0	60.7	15.0	43.3	30.5
1921	33.6	54.3	12.2	22.7	29.4	33.3	37.0
1920	58.8	46.3	2.6	36.3	30.3	16.0	10.0

Results in 1923 and 1924 are omitted, as yields at several stations were adversely affected by hail storms.

[1] *University of Alberta.*

[2] *University of Saskatchewan.*

TABLE II

YIELD, IN BUSHELS PER ACRE, OF WHEAT VARIETIES GROWN IN TEST PLOTS

Station	Variety	1931	1930	1929	1928	1927	1926	1925
Edmonton	Garnet	48.5	28.2	24.3	47.4	51.9	50.4	56.7
	Reward	40.3	26.5	18.3	45.6	46.9	46.3	45.8
	Red Fife	57.1	41.4	36.9	39.6	47.7	41.7	43.2
Lacombe	Garnet	67.2	38.8	41.1	41.3	48.0	48.8	42.0
	Reward	64.6	34.7	35.8	42.5	41.0	45.0	35.6
	Kitchener*	47.2	49.8	45.7	29.6	46.0	55.0	56.0
Lethbridge	Garnet	20.7	20.3	28.3	37.9	18.8	24.4	25.0
	Reward	19.1	17.6	27.0	35.5	19.8	23.9	25.0
	Red Fife	15.8	25.0	22.5	36.1	29.6	27.2	34.9
Indian Head	Garnet	7.3	40.8	28.3	34.7	30.0	57.5	48.8
	Reward	7.5	35.6	25.4	32.9	19.1	45.8	41.9
	Red Fife	19.8	29.8	32.5	37.3	36.1	49.1	41.2
Swift Current**	Garnet	6.9	25.7	20.1	45.7	39.1	31.1	26.5
	Reward	6.7	20.0	22.1	34.6	34.4	29.5	22.0
	Marquis	9.4	24.4	18.5	42.0	39.3	31.0	23.3
	Red Fife	9.4	24.2	17.7	44.8	37.4	34.0	28.0
Scott	Garnet	29.4	43.9	15.7	42.8	44.0	30.9	38.4
	Reward	23.5	38.9	18.1	43.3	39.0	28.0	36.4
	Red Fife	30.4	38.4	17.3	33.1	42.3	22.7	33.7
Rosthern	Garnet	13.8	29.2	44.8	27.5	34.0	20.0	28.3
	Reward	12.7	26.6	34.9	23.3	33.7	20.8	24.0
	Red Fife	18.3	29.9	50.9	28.8	28.0	18.3	33.3

**A late maturing variety; Red Fife not grown.*

***Station established in 1921.*

the annual yields (usually of Marquis, but in some cases of Reward or Garnet), listed in Table III, of the summerfallowed and stubble plots of a summerfallow-wheat-wheat rotation. Owing to its superior cultivation, the soil of the variety test plots will undoubtedly differ both chemically and physically from that of these grain rotation plots. In particular it may be expected to have a higher moisture-holding capacity, an increased proportion of fibrous constituents and greater amounts of readily nitrifiable plant residues and of plant nutrients of all kinds.

TABLE III

YIELD OF WHEAT, IN BUSHELS PER ACRE, FROM SUMMERFALLOWED AND STUBBLE LAND

Year	Lacombe		Lethbridge		Indian Head		Swift Current		Scott		Rosthern	
	Fallow	Stubble	Fallow	Stubble	Fallow	Stubble	Fallow	Stubble	Fallow	Stubble	Fallow	Stubble
1931	31.0	23.0	15.7	5.1	11.5	0.0	3.0	0.2	33.0	12.3	34.0	15.2
1930	17.6	8.8	28.5	12.1	24.2	23.1	19.0	5.1	35.8	26.1	26.4	23.1
1929	9.6	7.7	26.2	25.6	18.3	0.0	9.5	4.5	7.7	5.8	22.1	17.3
1928	33.0	28.0	52.1	27.1	32.7	19.5	32.1	18.9	37.0	23.8	20.8	19.3
1927	33.5	16.0	38.4	32.3	33.6	18.3	42.7	34.3	36.0	27.2	30.8	31.2
1926	34.5	26.0	28.2	29.7	30.8	25.4	20.5	12.5	18.8	10.9	30.4	20.0
1925	24.2	16.6	25.4	12.5	29.2	25.1	20.5	18.5	32.9	29.9	34.8	32.8
1922					34.5	18.6			13.3	12.2		
1921					31.6	24.3			24.3	24.0		
1920					14.6	5.2			25.0	18.0		

The meteorological data used, consisting of daily observations of precipitation and temperature, were extracted from the Monthly Record published by the Meteorological Service of Canada. Certain gaps occurred in the printed records but the information represented by these was supplied by the Director of the Meteorological Service.

3. Method of Analysis

The considerations outlined in Section 1 necessitate the determination of the effect on yield of any weather factor, at different periods of the growth cycle; and if a search is to be made for "critical periods" of possibly short duration, the season must be fairly finely subdivided. Five-day intervals have been suggested for this purpose. The amount of precipitation, etc., in each period may then be regarded as constituting an individual variable, the effect of which upon yield may be determined by the methods of partial correlation.

This direct analysis suffers, however, from serious disadvantages (12). The arithmetical work incidental to the formulation and solution of the necessary sets of simultaneous equations in 25 or more unknowns is extremely laborious. More important, however, is the consideration that owing to the high proportion of independent variables to observations, the standard errors of the regression coefficients will in all probability be so large as to destroy their significance.

These difficulties were overcome by Fisher (10), who introduced the concept of the *regression integral*, involving a regression function varying continuously with the time. The practical application of this method of analysis consists

in expressing the amount and distribution of, for example, rainfall in each year by means of the six numerical coefficients of a fifth-degree polynomial *function of the time;*

$$\rho_0 T_0 + \rho_1 T_1 + \rho_2 T_2 + \rho_3 T_3 + \rho_4 T_4 + \rho_5 T_5$$

the six terms T_r of order 0, 1, 2 . . . 5, being mutually orthogonal. The six coefficients ρ (found by Least Squares) corresponding to each year are then employed as independent variables with which the crop is to be correlated, in place of the 25 or more actual values of rainfall by periods. Then by virtue of the orthogonal properties of the functions T_r employed, the regression integral of yield upon rainfall may itself be expanded as a fifth-degree polynomial function of the time involving the partial regression coefficients α of yield upon the distribution coefficients ρ, and the effect on the crop of an extra inch of rainfall at any period displayed.

This method has been employed in the present study. But, whereas Fisher, dealing with an autumn sown crop, took into consideration the rainfall sequence throughout a period of 366 days ending on the 31st of August of the year in which the crop was harvested, attention has here been confined to a period of 125 days commencing 15 days before the date of sowing each year. Instead of the 61 six-day intervals into which he grouped the daily records of rainfall for the determination of the six coefficients ρ of each season, 25 five-day intervals have been employed. Otherwise, the procedure is the same. The time of sowing, rather than any fixed calendar date, was used as a reference point because the former may vary considerably from year to year, and from district to district in the same year, in exceptional cases by as much as one month.

In Table IV will be found the 462 rainfall distribution coefficients appropriate to the various stations and seasons for which crop records are available.

TABLE IV

RAINFALL DISTRIBUTION COEFFICIENTS

Station and year		ρ_0	ρ_1	ρ_2	ρ_3	ρ_4	ρ_5
Edmonton	1931	2.772	1.138	−.740	−.543	−.190	−.208
	1930	1.398	.273	−.588	−.413	.042	.074
	1929	1.500	.450	−.119	−.784	−.153	.090
	1928	2.170	.504	−.849	−.649	.134	.660
	1927	1.996	−.204	−1.026	−.650	1.041	.668
	1926	1.962	.605	−.656	.304	.595	−.392
	1925	1.924	−.039	1.084	−.443	1.380	−.609
	1922	1.492	.463	.653	.420	.476	.225
	1921	1.894	1.087	−.358	−.065	−.038	−.227
	1920	2.246	−.250	−.862	.165	−.278	−.666
Lacombe	1931	2.592	1.634	−.741	−.868	.577	1.391
	1930	1.810	.575	.168	−.270	−.542	−.451
	1929	1.012	.098	.019	.208	.261	.275
	1928	2.368	.577	−1.228	.284	1.667	−.176
	1927	2.584	.160	−.867	−.438	.390	.722
	1926	2.250	.803	−.111	.962	.328	−.285
	1925	1.072	.140	−.361	.029	.319	.043
	1922	1.422	.291	.115	−.107	.477	.362
	1921	1.644	.387	−.128	−.243	−.443	.188
	1920	1.018	−.251	−.149	.008	.132	.202

TABLE IV—*Concluded*
RAINFALL DISTRIBUTION COEFFICIENTS

Station and year		p_0	p_1	p_2	p_3	p_4	p_5
Lethbridge	1931	.996	−.194	−.101	−.349	.394	−.230
	1930	1.318	.132	.143	.050	−.545	−.594
	1929	1.866	−.889	−.397	.686	−.191	.470
	1928	2.458	.552	−1.460	.453	1.822	.535
	1927	2.464	−1.002	.076	1.219	−1.207	.395
	1926	1.208	.622	−.241	−.269	−.251	−.195
	1925	1.640	−.061	.413	.217	1.351	.405
	1922	1.232	−.167	−.428	−.321	.570	.037
	1921	1.166	.123	−.309	−.310	−.193	.118
	1920	1.120	.608	.229	−.742	.177	.614
Indian Head	1931	.656	.602	.063	−.307	−.321	−.022
	1930	1.040	.084	−.491	.046	.058	.065
	1929	.968	−.132	−.269	−.228	.481	−.461
	1928	2.164	.020	−.767	−.767	1.172	.002
	1927	2.128	.192	−.953	−.355	−.179	.215
	1926	1.530	−.237	−.559	−.339	.051	−.252
	1925	1.018	.194	−.588	−.082	.633	.293
	1922	1.812	−.494	−.322	.131	−.105	.395
	1921	2.624	−.054	−.984	−.242	.496	−.916
	1920	1.942	.395	−.750	−.796	−.439	.443
Scott	1931	1.216	.644	−.501	−.787	.049	.284
	1930	1.204	.550	−.470	−.642	−.177	.357
	1929	.900	.222	−.523	−.140	.411	.203
	1928	1.378	.089	−.856	−.078	.464	−.135
	1927	1.626	−.058	−.284	−.060	.102	.437
	1926	1.442	−.182	−.125	.462	.144	−.460
	1925	1.892	.201	−.599	−.101	.153	.307
	1922	1.364	.419	1.022	1.246	.533	.762
	1921	1.310	−.335	.171	−.275	.204	.409
	1920	1.480	.599	−.446	−.911	−.262	−.140
Rosthern	1931	1.398	1.167	.239	−.379	−.181	.573
	1930	1.276	.627	−.258	−.252	−.304	.075
	1929	1.160	.269	−.649	.023	.584	.002
	1928	1.506	1.298	.043	−.108	.182	−.655
	1927	3.004	.460	−.866	−.822	.638	1.334
	1926	1.226	−.374	−.275	.539	−.057	−.182
	1925	2.094	.190	−.266	.146	.221	.957
	1922	1.826	.201	.448	1.045	−.343	−.074
	1921	1.824	.350	−.480	−.807	−.157	.210
	1920	1.354	.292	−.211	−.242	.050	.454
Saskatoon	1931	1.324	.621	−.313	−.466	.156	.653
	1930	1.298	.334	−.653	−.381	.074	.413
	1929	1.148	.102	−.495	−.037	.340	.256
	1928	2.250	1.065	−.766	−.556	.410	.007
	1927	2.192	.057	−.946	−.637	.495	.621
	1926	1.402	−.557	−.060	.142	.477	−.624
	1925	2.102	−.326	−.483	.114	1.041	−.828
	1922	1.354	.402	.441	.942	.105	.254
	1921	2.034	−.145	−.916	−.071	.123	−.181
	1920	1.232	.174	−.068	−.376	−.420	.008
Swift Current	1931	1.282	.577	−.342	−.469	−.129	−.002
	1930	1.570	−.087	−.138	.176	.799	1.062
	1929	1.464	.156	−.903	.028	.695	.179
	1928	1.750	.360	−1.063	−.386	.666	.107
	1927	2.442	−.210	.228	.402	−.734	.587
	1926	1.538	.397	−.877	−.296	.075	−.077
	1925	1.266	−.441	.195	−.458	.404	−.386

The six numbers constituting each horizontal line specify the amount and distribution of rain in each season. As it was necessary to calculate 77 sets of six coefficients using the same series of values of the polynomial functions, six series of 25 multipliers, consisting of the values of the terms $T_0, T_1, \ldots T_5$ for the successive periods of time were constructed as suggested by Tippett (20) so that multiplication of the amount of rainfall in each period by the corresponding term of the appropriate series and addition of the resulting products gave the distribution coefficient directly. This procedure facilitated the computation of the coefficients ρ, but even so the arithmetical work involved was considerable.

4. Regression of Yield on Rainfall During the Growing Season

Rainfall and Yield of Marquis Wheat

The coefficients of regression $\alpha_0, \alpha_1, \ldots \alpha_5$ of yield y on the rainfall distribution coefficients $\rho_0 \ldots \rho_5$ are to be obtained from the solution of the six simultaneous equations

$$
\begin{aligned}
\alpha_0 S(\rho_0^2) + \alpha_1 S(\rho_0\rho_1) + \alpha_2 S(\rho_0\rho_2) + \cdots + \alpha_5 S(\rho_0\rho_5) &= S(\rho_0 y) \\
\alpha_0 S(\rho_0\rho_1) + \alpha_1 S(\rho_1^2) + \alpha_2 S(\rho_1\rho_2) + \cdots + \alpha_5 S(\rho_1\rho_5) &= S(\rho_1 y) \\
\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \\
\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots\cdots \\
\alpha_0 S(\rho_0\rho_5) + \alpha_1 S(\rho_1\rho_5) + \alpha_2 S(\rho_2\rho_5) + \cdots + \alpha_5 S(\rho_5^2) &= S(\rho_5 y) \qquad (1)
\end{aligned}
$$

the quantities ρ and y being measured from their respective means and the summations extending over the whole of the observational series. In order to eliminate any bias resulting from differences in average soil fertility or rainfall conditions between stations, the quantities to be correlated will not be the values of y, ρ_0, ρ_1, etc., actually observed, but the amount by which these are in excess or defect of their mean value at the appropriate station during the period under consideration. The sums of squares and products in Equation (1) will therefore be of the form

$$SS(\rho_{rp} - \bar{\rho}_{rp})(\rho_{sp} - \bar{\rho}_{sp})$$

where $\bar{\rho}_{rp}$ is the mean value of ρ_r at station p, rather than

$$S(\rho_r - \bar{\rho}_r)(\rho_s - \bar{\rho}_s)$$

where ρ_r is the mean of all values of ρ_r, and will correspond to 63 of the total 69 degrees of freedom.

Computation of these quantities yields the following numerical values:

$$
\begin{aligned}
16.916160\alpha_0 + .704273\alpha_1 - 7.374067\alpha_2 - .153974\alpha_3 + 3.366082\alpha_4 & \\
+ 2.677181\alpha_5 &= 129.51124 \\
.704273\alpha_0 + 13.445682\alpha_1 - .966389\alpha_2 - 5.327921\alpha_3 + .238760\alpha_4 & \\
+ 2.090910\alpha_5 &= -46.54435 \\
-7.374067\alpha_0 - .966389\alpha_1 + 15.382760\alpha_2 + 4.591087\alpha_3 - 3.442486\alpha_4 & \\
- 2.087548\alpha_5 &= -130.18403 \\
-.153974\alpha_0 - 5.327921\alpha_1 + 4.591087\alpha_2 + 16.260811\alpha_3 + .035470\alpha_4 & \\
- 3.460086\alpha_5 &= 4.45425 \\
3.366082\alpha_0 + .238760\alpha_1 - 3.442486\alpha_2 + .035470\alpha_3 + 18.600138\alpha_4 & \\
+ .981074\alpha_5 &= 32.98832 \\
2.677181\alpha_0 + 2.090910\alpha_1 - 2.087548\alpha_2 - 3.460086\alpha_3 + .981074\alpha_4 &
\end{aligned}
$$

giving:

$$\alpha_0 = 5.16397 \qquad \alpha_3 = .80204$$
$$\alpha_1 = -3.74079 \qquad \alpha_4 = -.67708$$
$$\alpha_2 = -6.74085 \qquad \alpha_5 = -.93891$$

The significance of the correlation between yield and the rainfall coefficients may be investigated by determining the distribution of the total variance of yield, corresponding to 63 degrees of freedom, between the six degrees of freedom of the regression formula and the 57 degrees of freedom appropriate to deviations of the actual yield values from the regression formula. It is apparent from Table V that the regression formula has in fact accounted for more than a proportionate amount of the variance and a definite, though moderate degree of relationship between the variables in question is indicated.

TABLE V

DISTRIBUTION OF SEASONAL VARIANCE OF YIELD OF MARQUIS WHEAT

Variance due to	Degrees of freedom	Sum of squares	Mean square
Regression formula	6	1698.31	283.05
Deviations from regression formula	57	6370.15	111.75
Total	63	8068.46	—

In certain circumstances it is conceivable that the pooling of the seven sets of sums of squares and products derived from the different stations might not be valid. The incidence of rainfall at the various stations during the period under review is not entirely independent; there is, as might be expected, a certain intra-annual correlation between stations with respect to both its amount and distribution. If then any extraneous source of variation in yield is common to different stations in a given year, an error will be introduced into the regression formula deduced in the preceding paragraphs.

This point was investigated by analyzing the total seasonal variance and covariance of yield and the rainfall coefficients (63 degrees of freedom) into two portions, of which the first represented the average variance and covariance between years common to all stations (nine degrees of freedom) and the second represented the residual variance and covariance at each station each year (54 degrees of freedom). Two sets of the sums of squares and products form-

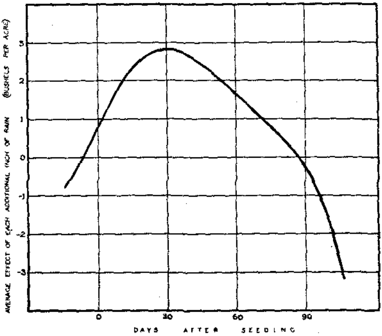

FIG 1. *Average effect of rainfall on yield of Marquis wheat (variety test plot data).*

ing the numerical coefficients of Equations (1) were thus obtained, yielding two series of values of the coefficients α. The differences between these were, however, insignificant when considered in relation to the residual deviations; consequently, the original regression formula, derived from the total 63 degrees of freedom, is employed in subsequent calculations.

As explained in Section 3, the values of α enable the average effect on the crop of an extra inch of rainfall at any time within the 125-day period to be estimated. Fig. 1 shows the course of the effect throughout that period.

Rainfall and Differential Yield of Wheat Varieties

For this study the crop yields of Table II are available, together with the corresponding yields of the variety Marquis from Table I. The quantities whose seasonal variation in excess or defect of their respective station averages is to be correlated with the rainfall coefficients are not the absolute yields, but the differences in yield between the varieties Garnet and Reward (both early maturing sorts), Garnet and Marquis (the latter medium-early maturing), Garnet and Red Fife (the latter late maturing), and Marquis and Red Fife. The resulting elimination of irregular variation due to differences in the fertility of the soil on which the trials were conducted in different years, common to both plots of any comparison, may be expected partially to offset the reduction in number of the observational series.

Owing to the smaller number of crop records, and the inclusion of results from the Swift Current station, the following new series of numerical values of the sums of squares and products of rainfall coefficients in Equations (1) must be computed:

12.441809	.447835	−4.232000	1.855544	2.699285	5.111341	
.447835	9.186527	−.422194	−3.748035	.565771	.077764	
−4.232000	−.422194	10.456264	.260742	−4.267647	−3.509379	
1.855544	−3.748035	.260742	7.905838	−.955070	−1.870688	
2.699285	.565771	−4.267647	−.955070	16.362681	1.468324	
5.111341	.077764	−3.509379	−1.870688	1.468324	10.407955	(3)

As it will eventually be necessary to solve equations involving the present set of values of these independent variates in conjunction with ten different dependent variates, it will be advantageous to calculate at this stage the values of

$$c_{rs} = \frac{\Delta_{rs}}{\Delta}$$

where Δ_{rs} is the co-factor of the element in the rth row and s^{th} column of the determinant formed from the matrix of the numerical coefficients of Equations (3). These c values, determined in the manner outlined by Fisher (11, Sec. 29) are given in Table VI. The partial regression coefficient α_s of any dependent variate y on ρ_s may then be rapidly obtained from the relation:

$$\alpha = c_{0s} S(\rho_0 y) + c_{1s} S(\rho_1 y) + \ldots\ldots\ldots\ldots + c_{5s} S(\rho_5 y) \qquad (4)$$

TABLE VI

VALUES OF c_{rs}

	$c_{\cdot 0}$	$c_{\cdot 1}$	$c_{\cdot 2}$	$c_{\cdot 3}$	$c_{\cdot 4}$	$c_{\cdot 5}$
$c_{0\cdot}$	.127657	−.028570	.026392	−.060570	−.011081	−.062904
$c_{1\cdot}$	−.028570	.143207	.002755	.081472	.002704	.028120
$c_{2\cdot}$	.026392	.002755	.125698	.000207	.026035	.025766
$c_{3\cdot}$	−.060570	.081472	.000207	.195697	.013039	.062541
$c_{4\cdot}$	−.011081	.002704	.026035	.013039	.069800	.006697
$c_{5\cdot}$	−.062904	.028120	.025766	.062541	.006697	.145746

TABLE VII

SUMS OF PRODUCTS OF VARIETAL YIELD DIFFERENCES AND RAINFALL COEFFICIENTS

	Garnet-Reward	Garnet-Marquis	Garnet-Red Fife	Marquis-Red Fife
$S(\rho_0 y)$	15.6465	22.7739	52.2249	32.1449
$S(\rho_1 y)$	−8.3329	7.2119	4.0452	−3.7745
$S(\rho_2 y)$	12.3720	−24.0684	−37.8503	−41.7687
$S(\rho_3 y)$	4.4511	11.9291	−4.9571	−12.4828
$S(\rho_4 y)$	−1.7251	42.6996	81.0705	33.2225
$S(\rho_5 y)$	1.2907	8.3294	31.2984	33.2491

Utilizing the differences in yield of the above-mentioned wheat varieties as successive dependent variates, the sums of products in Table VII and the following regression coefficients result:

Difference in yield of Garnet and Reward

$\alpha_0 = 2.23029$ $\qquad$ $\alpha_3 = -.69475$

$\alpha_1 = -1.21200$ $\qquad$ $\alpha_4 = .07248$

$\alpha_2 = 1.93438$ $\qquad$ $\alpha_5 = -.44483$

Difference in yield of Garnet and Marquis

$\alpha_0 = .34679$ $\qquad$ $\alpha_3 = 2.61538$

$\alpha_1 = 1.63877$ $\qquad$ $\alpha_4 = 2.33236$

$\alpha_2 = -1.07564$ $\qquad$ $\alpha_5 = .39608$

Difference in yield of Garnet and Red Fife

$\alpha_0 = 2.98575$ $\qquad$ $\alpha_3 = -.79706$

$\alpha_1 = -.31845$ $\qquad$ $\alpha_4 = 4.25054$

$\alpha_2 = -.45215$ $\qquad$ $\alpha_5 = .64787$

Difference in yield of Marquis and Red Fife

$\alpha_0 = 1.40547$ $\qquad$ $\alpha_3 = -2.19339$

$\alpha_1 = -1.56621$ $\qquad$ $\alpha_4 = .92497$

$\alpha_2 = -2.69321$ $\qquad$ $\alpha_5 = 1.08334$

The distribution of the seasonal variance of the varietal yield differences between the six degrees of freedom of the appropriate regression formula and the remaining 36 degrees of freedom is shown in Table VIII. In each case the proportion of the total variance which can be expressed by means of the

regression formula is low, and in only two instances does the regression mean square exceed the mean square deviation from the regression formula. Both of these involve the late-maturing variety Red Fife, but the actual ratios of the mean squares correspond to z values of only .216 and .279, respectively, whereas a value of .430 or more might be expected to occur on the average once in 20 times as the result of chance differences alone. It must be concluded therefore that the observed fluctuations in relative yield are not associated in any consistent manner with the rainfall conditions prevailing during the growing season.

TABLE VIII

DISTRIBUTION OF SEASONAL VARIANCE OF VARIETAL YIELD DIFFERENCES

Variance	Garnet-Reward	Garnet-Marquis	Garnet-Red Fife	Marquis-Red Fife
Sum of squares:				
Due to regression (6 degrees of freedom)	65.14	179.69	540.58	257.71
Deviations from regression (36 degrees of freedom)	518.18	1721.79	2104.05	884.94
Total (42 degrees of freedom)	583.32	1901.48	2644.63	1142.65
Mean square:				
Due to regression	10.86	29.95	90.10	42.95
Deviations from regression	14.39	47.83	58.45	24.58

Rainfall and Yield of Wheat from Summerfallowed and Stubble Land

The crop data to be used in this study are those of Table III. Inspection reveals that the level of yield is definitely higher on the fallowed than on the stubble plots, but that both are inferior in this respect to the variety test plots. This point is illustrated in Fig. 2, in which the mean yield and seasonal standard deviation of both series are shown, together with the corresponding values deduced from the Marquis yields of Table I. In spite of the increased average yield, the annual fluctuations of the summerfallowed are no less, but rather slightly greater, than those exhibited by the stubble land crops. The extra moisture and plant nutrients accumulated as a result of the fallow are reflected in the higher level of yield, but apparently have not sufficed to exert any absolute stabilizing effect, though the percentage variation is lower.

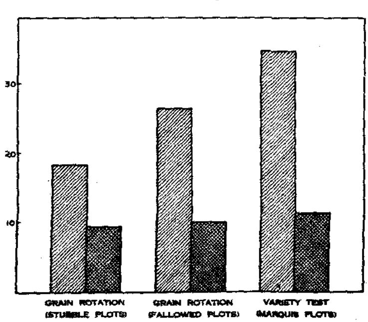

FIG. 2. *Average yield (single-hatched column) and annual variation (cross-hatched) in bushels per acre of three series of wheat plots.*

The numerical values of the sums of squares and products of rainfall coefficients in Equations (1) corresponding to the years and stations for which crop yields are available in Table III are:

12.843475	−.335858	−4.365599	1.826327	1.794788	4.455203	
−.335858	8.950589	−.005577	−4.254751	1.254894	.280159	
−4.365599	−.005577	9.856559	2.416812	−4.985248	−.724501	
1.826327	−4.254751	2.416812	10.123139	−.217015	−.367921	
1.794788	1.254894	−4.985248	−.217015	15.041716	1.515575	
4.455203	.280159	−.724501	−.367921	1.515575	10.644241	(5)

From these, the quantities c_{rs} shown in Table IX may be obtained in the manner already indicated. Multiplication of the six values in each column of Table IX by the corresponding six sums of products from Table X, as in Equation (4) page 314, yields the following regression coefficients of yield on the rainfall coefficients:

TABLE IX

VALUES OF c_{rs}

	$c_{\cdot 0}$	$c_{\cdot 1}$	$c_{\cdot 2}$	$c_{\cdot 3}$	$c_{\cdot 4}$	$c_{\cdot 5}$
$c_{0\cdot}$	.123494	−.018813	.070191	−.048457	.014451	−.050149
$c_{1\cdot}$	−.018813	.149510	−.037348	.074932	−.022246	.007155
$c_{2\cdot}$	.070191	−.037348	.175304	−.070012	.054516	−.026646
$c_{3\cdot}$	−.048457	.074932	−.070012	.156038	−.023670	.022308
$c_{4\cdot}$	.014451	−.022246	.054516	−.023670	.085830	−.014792
$c_{5\cdot}$	−.050149	.007155	−.026646	.022308	−.014792	.115813

Yield from stubble land:

$\alpha_0 = 9.09916$ $\qquad \alpha_3 = .83645$

$\alpha_1 = -2.34024$ $\qquad \alpha_4 = -1.61300$

$\alpha_2 = -2.37545$ $\qquad \alpha_5 = .03891$

Yield from summerfallowed land:

$\alpha_0 = 9.80077$ $\qquad \alpha_3 = .00891$

$\alpha_1 = -.48082$ $\qquad \alpha_4 = -.70476$

$\alpha_2 = -3.87536$ $\qquad \alpha_5 = .19378$

Difference in yield from summerfallowed and stubble land:

$\alpha_0 = .70161$ $\qquad \alpha_3 = -.82754$

$\alpha_1 = 1.85942$ $\qquad \alpha_4 = .90824$

$\alpha_2 = -1.49991$ $\qquad \alpha_5 = .15488$

It will be observed from Table XI that an appreciable proportion of the annual variance of yield of both the stubble and summerfallowed plots is accounted for by the appropriate regression formula. The ratio of the mean square due to the regression to the mean square deviation

TABLE X

SUMS OF PRODUCTS OF YIELD AND RAINFALL COEFFICIENTS

	Stubble yields	Summer-fallow yields	Difference in yield
$S(\rho_0 y)$	126.7605	142.5702	15.8097
$S(\rho_1 y)$	−29.6080	−8.4421	21.1659
$S(\rho_2 y)$	−52.9048	−77.5864	−24.6816
$S(\rho_3 y)$	29.6453	10.7511	−18.8942
$S(\rho_4 y)$	.2946	25.9970	25.7024
$S(\rho_5 y)$	39.2906	47.3286	8.1190

TABLE XI

DISTRIBUTION OF SEASONAL VARIANCE OF YIELD FROM SUMMERFALLOWED AND STUBBLE LAND

Variance	Stubble yields	Summer-fallow yields	Difference in yield
Sum of squares:			
Due to regression (6 degrees of freedom)	1374.22	1692.98	127.71
Deviations from regression (36 degrees of freedom)	2331.27	2498.29	1566.65
Total (42 degrees of freedom)	3705.49	4191.27	1694.36
Mean square:			
Due to regression	229.04	282.16	21.28
Deviations from regression	64.76	69.40	43.52

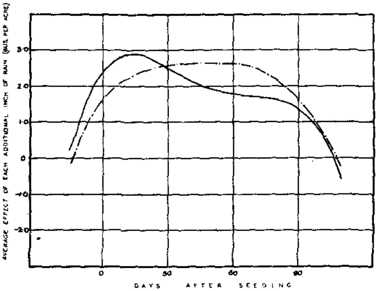

FIG. 3. *Average effect of rainfall on yield of wheat from stubble plot (solid line) and summerfallowed plot (broken line) of grain rotation.*

corresponds to a z value of .632 and .701 in the case of the stubble and fallow series, respectively, both being in excess of the 1% point, .623. The regression of the difference in yield in the various seasons between the summerfallowed and stubble plots on the rainfall coefficients is, however, totally insignificant. Fig. 3 shows the course of the two regression functions throughout the season.

Discussion of Results

The results as a whole may be held to reflect clearly the sub-optimal moisture conditions prevailing during the average western Canadian season. Certainly the curves in Figs. 1 and 3 differ markedly from the corresponding portions of those obtained by Fisher (10) for winter wheat and by Wishart and Mackenzie (21) for spring-sown barley under English conditions, with which they may be compared by taking the average date of seeding to be May 1.

The detrimental effect of rain later than 90 days after sowing, a pronounced feature of Fig. 1, is not reproduced in either of the curves in Fig. 3 and may probably be ascribed to loss of crop through lodging of the longer-strawed plants produced on the more fertile variety test plots. It is interesting to note that Fisher found the dunged plot *2b* to differ from all the other Broadbalk plots in the severe damage caused by rain immediately preceding harvest. A similar adverse effect manifests itself on all the Hoos field barley plots receiving nitrogenous dressings (21), but is biggest and earliest in the case of the farmyard manure plot.

It should be emphasized in any discussion of Figs. 1 and 3 that the curves indicate not the direct effect of so much rain, but the resultant of the entire combination of weather conditions, such as low temperature, high relative humidity, lack of sunshine, etc., associated with rainfall at any given time. Thus the detrimental effect on the varietal plot yields of rainfall prior to sowing may be due largely to the accompanying reduction in temperature.

The lack of correlation between varietal differences and the rainfall coefficients does not necessarily mean that the former are unaffected by weather conditions, but does indicate that there is no consistent relation between the observed differences (which are sometimes large) and the rainfall sequence during the growing season. Such inconsistencies may be attributable to any or all of a variety of factors, *e.g.*, other meteorological phenomena not associated with rainfall, weather conditions (including precipitation) during the preceding summer, autumn and winter, and soil differences both within and between stations.

Considering the season as a whole, both the summerfallowed and stubble plots of the grain rotation show a greater response to rainfall than do the variety test plots. This is particularly the case in the later stages of growth. The enhanced moisture-holding capacity of the more fertile soil of the variety plots may result in a larger reserve supply of moisture still available at this time to meet the requirements of the crop, which, as already noted, was found by Briggs and Shantz (6) to attain its maximum rate of transpiration prior to the onset of ripening. It is well known, moreover, (*e.g.*, 15, 18) that the degree of fertility of the soil has a marked effect on the efficiency of transpiration, with the result that the water requirement on a poor soil is considerably higher than on a fertile one. There is some indication (Fig. 3) that the stubble plots of the grain rotation are more responsive to wet weather in the early part of the season and that the fallowed plots are able to utilize later rains more advantageously, perhaps owing in the latter case to a more extensive plant development in the preceding period made possible by greater reserves of moisture and nutrients. As, however, these differences are not at any time pronounced, and are statistically quite insignificant, such considerations cannot be more than conjectural.

The maximum benefit from additional rain on the variety plots is obtained approximately one month after the date of sowing. About this time tillering is taking place and a period of active growth is being initiated. It is possible, therefore, that additional moisture in the surface level induces a more prolific vegetative growth, which the fertile soil is capable of reflecting in increased yields. Smith (19), for example, found from 8000 observations on 64 varieties of spring wheat, oats and barley grown in a cereal nursery at Dickinson, N. Dakota, a high correlation between rainfall from May 16 to July 15, extent of tillering, and yield in different seasons, and concluded that under local conditions there was a close relation between rainfall and tillering and between tillering and yield.

The degree of correlation between seasonal yield and the rainfall coefficients is only moderate, being represented by values of $r = 0.46$, 0.60 and 0.62 in the case of the variety test (Marquis), stubble and summerfallow series, respectively. It should be recognized that the residual variance, not accounted for by the regression formulas, may arise not only from the operation of all other meteorological factors not associated with rainfall, and of weather conditions antecedent to the growing season, but also from variations introduced by growing the crop on different land each season (particularly in the case of the variety plots) and from dissimilarities between the true values of the regression function at different stations. Such variance may thus in part reflect the imperfections of the data available rather than an actual absence of causal relation.

5. Characteristics of Seasonal Rainfall

The extent to which the annual yields are influenced by the rainfall conditions in any particular period will depend not only on the effect of a given increment of rain at that time, but also on the variability of the amount of rain actually falling during this period from year to year. A study has therefore been made of the seasonal rainfall at several meteorological stations in central and southern Saskatchewan and Alberta, the results of which are presented graphically in Fig. 4. With the exception of Lacombe (no records prior to 1908), data for the years 1900-1931, inclusive, were available from the following stations:

Central Alberta: Edmonton, Lacombe.
Central Saskatchewan: Battleford, Saskatoon.
Southern Alberta: Calgary, Lethbridge.
Southern Saskatchewan: Indian Head, Swift Current.

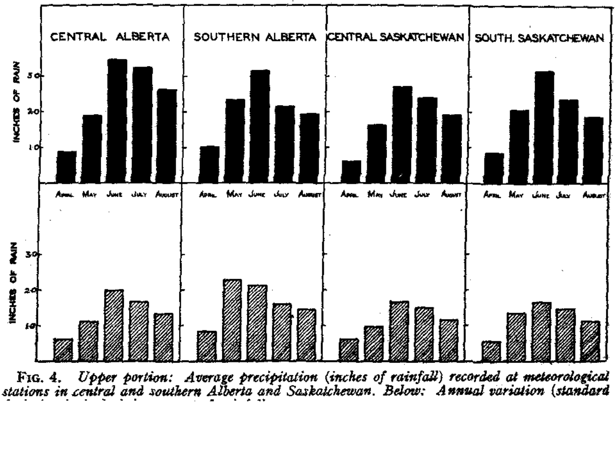

FIG. 4. *Upper portion: Average precipitation (inches of rainfall) recorded at meteorological stations in central and southern Alberta and Saskatchewan. Below: Annual variation (standard*

The upper portion of Fig. 4 portrays the average rainfall in each of the five months April-August, inclusive, recorded at the stations in these districts. The average rainfall sequence is similar in all districts, progressing from the low value of one inch or less in April to a maximum of approximately three inches in June. Seasonal fluctuations in the amount of rain received in the various months are measured by the standard deviation of the monthly totals, shown in the lower part of the diagram. These also follow a similar course in all districts except southern Alberta, where the variability of May rainfall exceeds that of June and July, otherwise uniformly the most variable months. The uncertainty of the rainfall in these regions is well illustrated by the high ratio of the standard deviations to the corresponding average monthly totals.

Referring to Figs. 1 and 3 and taking the average date of sowing to be May 1, the beneficial effect of additional rain on both the variety test plots and the fallowed plots of the grain rotation is seen to be greatest during the month of June. As this month also exhibits the greatest variability in amount of rain, June precipitation appears to exert a greater influence upon the yield of these plots than that of any other month. Seasonal differences in July and August rainfall may also affect appreciably the yield secured from the variety test and summerfallowed grain rotation plots, respectively. May rainfall is in three out of the four cases more constant from year to year but, if deficient, may be expected to reduce the yield of the stubble plots and of the variety test plots.

The seasonal incidence of precipitation in the four districts may be investigated by averaging the total amounts recorded during the five months at the two meteorological stations in each district. The mean values and standard deviations of these averages are:

	Central Alberta	Southern Alberta	Central Saskatchewan	Southern Saskatchewan
Mean (inches)	12.3	10.6	9.2	10.3
Standard deviation	2.76	4.03	2.57	2.71

Table XII gives the coefficients of correlation calculated from the amounts of rain recorded in different districts during the same season. Five of the six coefficients are significant, showing that the rainfall in the four districts during a given season is not independent. The degree of association indicated, however, is not as high as might perhaps have been anticipated between the precipitation in adjacent parts of a comparatively

TABLE XII

Correlation of seasonal rainfall in four districts

	Southern Alberta	Central Saskatchewan	Southern Saskatchewan
Central Alberta	.43	.45	.24
Southern Alberta		.43	.55
Central Saskatchewan			.56

Value of r for {*5% level of significance = .35*; *1% level of significance = .45*}

unbroken area such as the Canadian plains, with the result that the simultaneous occurrence of extremely dry or wet seasons over the whole area may be expected to be infrequent.

6. Additional Effect of Temperature

The regression of wheat yield on rainfall having been found, the partial regression of yield on temperature, eliminating all associated rainfall effect, may be determined in the manner outlined by Tippett (20). The temperature sequence of each season is first expressed as a continuous function of the time, using the orthogonal polynomial functions of zero, first, second, etc., order in time already employed in the reduction of the rainfall data. In this way a series of sets of six numerical coefficients $\theta_0, \theta_1, \ldots, \theta_5$, representing the temperature conditions of different seasons, is obtained. The regression of each of the temperature coefficients on the six rainfall coefficients is next found, and the residual deviations of the temperature coefficients are then correlated with the corresponding residual deviations of wheat yield from the rainfall regression formulas of Section 4.

As an index of the prevailing temperature conditions the daily means estimated from maximum and minimum thermometer readings have been used. The imperfections of such estimates in comparison with those to be obtained from a continuous record, or from thermometer readings at regular intervals, is well known, but more precise observations are not available.

From the observed daily temperatures the numerical values of the coefficients θ were computed by the procedure used in obtaining the rainfall coefficients of Section 3, and are listed in Table XIII. Owing to the inequality of the number of seasons in the three series of crop yields, the regression of the temperature coefficients on the rainfall coefficients of each series must be determined separately.

TABLE XIII

TEMPERATURE DISTRIBUTION COEFFICIENTS

Station and year		θ_0	θ_1	θ_2	θ_3	θ_4	θ_5
Edmonton	1931	2815	252	−106	53	−46	66
	1930	2729	413	−11	−34	40	−10
	1929	2746	347	−137	15	30	−75
	1928	2872	166	−133	−24	−102	−35
	1927	2783	322	−127	−38	17	−35
	1926	2830	228	−57	−118	−98	26
	1925	2848	343	−121	−33	−78	−39
	1922	2844	318	−176	−6	−65	11
	1921	2813	291	−146	−68	40	−32
	1920	2831	268	−188	−99	−47	120
Lacombe	1931	2650	323	−76	−46	9	−113
	1930	2698	364	−8	−59	−2	19
	1929	2666	408	−99	−8	3	−37
	1928	2772	272	−168	41	−158	6
	1927	2750	304	−126	−25	29	−38
	1926	2728	126	−112	−121	15	97
	1925	2824	296	−117	−54	−29	−24
	1922	2695	419	−156	74	4	14
	1921	2721	322	−111	−57	29	−36

TABLE XIII—*Concluded*

TEMPERATURE DISTRIBUTION COEFFICIENTS

Station and year		θ_0	θ_1	θ_2	θ_3	θ_4	θ_5
Lethbridge	1931	2781	392	−49	−7	41	−43
	1930	2669	382	34	−4	−80	37
	1929	2766	458	−106	31	−30	−13
	1928	2921	146	−73	−83	−111	−7
	1927	2809	286	−129	−63	88	−34
	1926	2902	186	−54	−137	−70	14
	1925	2853	329	−155	−44	11	27
	1922	2913	285	−146	10	−7	−25
	1921	2928	278	−202	−8	24	9
	1920	2846	459	−143	−70	−78	41
Indian Head	1931	2800	533	−33	−78	5	34
	1930	2681	449	66	−137	−21	8
	1929	2623	617	−122	−44	−48	16
	1928	2761	479	−220	162	−112	31
	1927	2697	453	−165	−71	6	86
	1926	2683	531	−146	182	−147	29
	1925	2784	371	−71	−141	39	−83
	1922	2967	200	−107	3	−34	−112
	1921	2923	291	−279	−18	66	43
	1920	2922	351	−143	22	−22	117
Scott	1931	2766	361	−157	−45	2	−56
	1930	2692	409	−58	−60	46	−25
	1929	2642	474	−154	−20	−5	−35
	1928	2802	290	−194	60	−142	51
	1927	2760	266	−130	−71	85	7
	1926	2782	233	−79	−115	−128	25
	1925	2703	368	−84	−98	10	−54
	1922	2746	406	−101	66	−3	−6
	1921	2849	307	−220	−43	33	24
	1920	2765	456	−123	29	−76	22
Rosthern	1931	2733	474	−58	−22	−6	37
	1930	2739	478	−44	−95	24	42
	1929	2795	432	−158	56	25	−76
	1928	2797	401	−224	106	−124	−11
	1927	2752	426	−141	−37	−16	23
	1926	2847	359	−75	−27	−142	29
	1925	2793	390	−75	−86	54	−64
	1922	2931	342	−121	66	−46	−21
	1921	2918	295	−250	−32	5	28
	1920	3002	280	−143	−52	−15	106
Saskatoon	1931	2804	352	−36	−49	7	−23
	1930	2754	444	20	−121	58	9
	1929	2759	481	−144	−32	16	−22
	1928	2875	315	−220	103	−152	60
	1927	2872	288	−132	−21	92	−5
	1926	2883	215	−126	−136	−43	50
	1925	2904	324	−190	−10	−12	15
	1922	2898	414	−132	47	−20	43
	1921	2939	319	−240	−58	30	33
	1920	2900	398	−211	−48	−70	13
Swift Current	1931	2947	365	−88	−76	63	−62
	1930	2852	404	30	−118	34	13
	1929	2894	471	−89	−46	35	−52
	1928	2936	399	−171	161	−123	74
	1927	3084	354	−200	−107	125	59
	1926	2920	384	−30	71	−173	19
	1925	2964	350	−136	−80	31	−1

Temperature and Yield of Marquis Wheat

In order to determine the regression of the temperature coefficients on the rainfall coefficients, the sums of products of the seasonal deviations of these two sets of quantities from their average values at the various stations must be found. These sums of products are given in Table XIV. Together with the corresponding sums of squares and products of the rainfall coefficients in the left-hand side of Equations (2), they provide the necessary data for the calculation of the required regression coefficients, which have the values indicated in Table XV.

TABLE XIV

SUMS OF PRODUCTS OF SEASONAL DEVIATIONS OF RAINFALL AND TEMPERATURE COEFFICIENTS (SERIES I)

	θ_0	θ_1	θ_2	θ_3	θ_4	θ_5
ρ_0	428.7280	−1239.4226	−650.8052	366.5496	−11.0012	164.9752
ρ_1	−177.4032	135.4570	291.2175	156.0703	−217.5229	12.7797
ρ_2	−150.8797	746.1933	307.1580	160.0163	84.3347	−63.3566
ρ_3	78.4404	−535.4481	129.0684	−124.1476	−150.7129	14.8424
ρ_4	145.7326	−322.5385	−510.7768	275.8483	−525.8077	−226.1613
ρ_5	−167.5631	248.8022	316.1284	−89.4370	625.4040	−500.9116

TABLE XV

REGRESSION OF TEMPERATURE COEFFICIENTS ON RAINFALL COEFFICIENTS (SERIES I)

	θ_0	θ_1	θ_2	θ_3	θ_4	θ_5
ρ_0	29.83306	−63.22830	−48.55154	39.03065	− 4.73207	14.47272
ρ_1	−13.30091	−3.53529	28.07211	7.77900	−27.85799	3.67032
ρ_2	1.59389	33.03970	.79859	29.12029	18.11712	2.96908
ρ_3	−3.12282	−38.48140	23.26893	−15.80847	−13.48704	−7.536429
ρ_4	−3.62326	.22028	21.92718	−14.95925	27.58732	15.00287
ρ_5	−16.09770	26.00405	32.27009	−13.64222	47.55967	−41.90966

It is not necessary to calculate the actual values of the individual deviations in order to determine the regression of the yield residuals on the temperature residuals. The required sums of squares and products of these quantities may be obtained from the corresponding values prior to the fitting of the rainfall regression by means of the relations:

$$S(\theta_r - \beta_{r0}\rho_0 - \beta_{r1}\rho_1 - \;\ldots\; - \beta_{r5}\rho_5)^2 = S(\theta_r^2) - \beta_{r0}S(\theta_r\rho_0) - \beta_{r1}S(\theta_r\rho_1) - \;\ldots\; - \beta_{r5}S(\theta_r\rho_5) \qquad (6)$$

$$S(\theta_r - \beta_{r0}\rho_0 - \beta_{r1}\rho_1 - \;\ldots\; - \beta_{r5}\rho_5)(\theta_s - \beta_{s0}\rho_0 - \beta_{s1}\rho_1 - \;\ldots\; - \beta_{s5}\rho_5) = S(\theta_r\theta_s) - \beta_{r0}S(\theta_s\rho_0) - \beta_{r1}S(\theta_s\rho_0) - \;\ldots\; - \beta_{r5}S(\theta_s\rho_5) \qquad (6a)$$

where β_{rs} is the regression coefficient of θ_r on ρ_s.

The seasonal variance and covariance of yield and the temperature coefficients provide the following sums of squares and products:

	θ_0	θ_1	θ_2	θ_3	θ_4	θ_5	Yield
θ_0	421227.6	−296405.0	−138159.2	−1075.5	−18083.1	29596.2	8887.49
θ_1	−296405.0	524177.7	85035.5	87427.9	30509.1	−31833.7	−23447.17
θ_2	−138159.2	85035.5	295441.8	−112294.4	42250.4	−45360.9	2117.49
θ_3	−1075.5	87427.9	−112294.4	305773.5	−106631.8	−3234.2	−1418.98
θ_4	−18083.1	30509.1	42250.4	−106631.8	260322.8	−58200.9	−1673.04
θ_5	29596.2	−31833.7	−45360.9	−3234.2	−58200.9	160786.1	−4472.18

Adjusting these by means of (6) and (6a) gives the following equations to determine the regression coefficients γ of the yield on the temperature residuals:

$$404393.8\gamma_0 - 257652.5\gamma_1 - 111718.5\gamma_2 - 11017.9\gamma_3 - 13090.8\gamma_4 + 16108.8\gamma_5 = 4871.30$$

$$-257652.5\gamma_0 + 394632.4\gamma_1 + 31626.0\gamma_2 + 103356.7\gamma_3 + 4310.6\gamma_4 + 5617.4\gamma_5 = -11065.47$$

$$-111718.5\gamma_0 + 31626.0\gamma_1 + 253418.9\gamma_2 - 99390.7\gamma_3 + 42515.4\gamma_4 - 16038.2\gamma_5 = 8485.58$$

$$-11017.9\gamma_0 + 103356.7\gamma_1 - 99390.7\gamma_2 + 286537.0\gamma_3 - 108490.0\gamma_4 - 18409.5\gamma_5 = -1446.99$$

$$-13090.8\gamma_0 + 4310.6\gamma_1 + 42515.4\gamma_2 - 108490.0\gamma_3 + 234522.0\gamma_4 - 25653.8\gamma_5 = -1509.39$$

$$16108.8\gamma_0 + 5617.4\gamma_1 - 16038.2\gamma_2 - 18409.5\gamma_3 - 25653.8\gamma_4 + 141051.0\gamma_5 = -6338.72$$

The values of γ satisfying these equations are:

$$\gamma_0 = .0064312 \qquad \gamma_3 = .0150464$$
$$\gamma_1 = -.0306698 \qquad \gamma_4 = -.0108923$$
$$\gamma_2 = .0445184 \qquad \gamma_5 = -.0394075$$

The effect on the crop of a given increment of temperature at different times throughout the season may be deduced from these coefficients as explained in Section 3, and is illustrated in Fig. 5. Table XVI shows the proportion of the variance of the yield residuals expressible by the temperature regression formula. This is seen to be only moderate, and even though the greater part of it is in fact attributable to the terms of first and second degree, the significance of the estimated effects is in some doubt.

TABLE XVI

DISTRIBUTION OF SEASONAL VARIANCE OF YIELD OF MARQUIS WHEAT

Variance due to	Degrees of freedom	Sum of squares	Mean square
Rainfall regression formula	6	1698.31	283.05
Residual temperature regression formula	6	992.93	165.49
Deviations from regression formulas	51	5377.22	105.43
Total	63	8068.46	—

Temperature and Differential Yield of Wheat Varieties

The seasonal covariance of the rainfall and temperature coefficients during the period for which varietal comparisons are available is productive of the sums of products listed in Table XVII. Multiplication of these by the successive columns of c values of Table VI as indicated in Equation (4), page 314, enables the regression of the seasonal fluctuations of the temperature coefficients on those of the rainfall coefficients to be readily determined, the numerical values being given in Table XVIII. Tables XVII and XVIII enable the sums of squares and products of the seasonal deviations of the temperature coefficients to be adjusted to give the corresponding functions of the residuals, required for the determination of the regression of the yield residuals on the temperature residuals. The unadjusted values are:

	θ_0	θ_1	θ_2	θ_3	θ_4	θ_5
θ_0	221797.8	−124098.4	−51633.4	−31824.8	−14355.6	12472.0
θ_1	−124098.4	269063.8	30872.4	79153.2	55718.1	−36307.3
θ_2	−51633.4	30872.4	184827.8	−98700.8	32392.8	−12016.8
θ_3	−31824.8	79153.2	−98700.8	271426.7	−149209.5	25215.5
θ_4	−14355.6	55718.1	32392.8	−149209.5	252250.8	−54108.7
θ_5	12472.0	−36307.3	−12016.8	25215.5	−54108.7	99198.7

The residual sums of squares and products obtained from these by means of (6) and (6a), page 324, are:

	θ_0	θ_1	θ_2	θ_3	θ_4	θ_5
θ_0	204048.9	−96491.7	−27884.0	−45544.9	6142.4	−3898.0
θ_1	−96491.7	191653.0	10222.5	74448.6	16047.2	−5523.7
θ_2	−27884.0	10222.5	131552.5	−48547.1	−9461.7	7645.8
θ_3	−45544.9	74448.6	−48547.1	174538.0	−75222.7	9732.0
θ_4	6142.4	16047.2	−9461.7	−75222.7	128662.1	−22180.9
θ_5	−3898.0	−5523.7	7645.8	9732.0	−22180.9	72142.3

TABLE XVII

Sums of products of seasonal deviations of rainfall and temperature coefficients (Series II)

	θ_0	θ_1	θ_2	θ_3	θ_4	θ_5
ρ_0	375.9597	−633.5518	−548.8587	220.6140	−55.3437	237.2455
ρ_1	86.8920	−279.2813	163.4911	31.9991	−207.7983	45.3690
ρ_2	−53.2085	366.5013	198.1474	−318.5033	478.7752	43.7068
ρ_3	92.6571	−422.6514	−29.3746	−409.6782	−10.3239	230.1808
ρ_4	216.3768	−227.0621	−454.6179	279.1454	−665.9853	−36.7730
ρ_5	−27.5618	14.9081	56.4651	−320.2172	689.6705	−320.8584

TABLE XVIII

Regression of temperature coefficients on rainfall coefficients (Series II)

	θ_0	θ_1	θ_2	θ_3	θ_4	θ_5
ρ_0	37.83086	−36.04711	−66.24214	60.70666	−23.87033	36.79157
ρ_1	8.91464	−55.51375	37.60539	−44.22227	−10.10621	9.47086
ρ_2	8.41584	22.96345	.48301	−35.19257	58.57674	2.70329
ρ_3	3.52677	−69.04345	38.46038	−107.38116	18.95003	13.83466
ρ_4	10.81043	−5.45320	−20.05465	1.34768	−29.48537	−3.08344
ρ_5	−19.34992	15.66161	47.57602	−91.60699	105.38554	−45.13542

In the same way, the sums of products of yield and temperature residuals, shown in Table XX, may be obtained from the unadjusted quantities of Table XIX. The regression of the four series of yield-difference residuals on the temperature residuals may then be deduced, the regression coefficients γ taking the values shown below:

TABLE XIX

SUMS OF PRODUCTS OF VARIETAL YIELD DIFFERENCES AND TEMPERATURE COEFFICIENTS

	Garnet-Reward	Garnet-Marquis	Garnet-Red Fife	Marquis-Red Fife
$S(\theta_0 y)$	2637.48	−2909.42	1516.18	4010.90
$S(\theta_1 y)$	−1226.75	530.76	−4553.94	−4322.18
$S(\theta_2 y)$	−2176.92	−212.75	−582.83	−492.84
$S(\theta_3 y)$	549.25	1150.77	800.51	420.69
$S(\theta_4 y)$	1574.68	−4817.03	−8068.49	−3050.12
$S(\theta_5 y)$	1063.25	−1808.47	−2956.67	−663.46

TABLE XX

SUMS OF PRODUCTS OF VARIETAL YIELD DIFFERENCE RESIDUALS AND TEMPERATURE RESIDUALS

	Garnet-Reward	Garnet-Marquis	Garnet-Red Fife	Marquis-Red Fife
$S(\theta_0 y)$	2043.65	−3975.51	−490.39	3470.64
$S(\theta_1 y)$	−1131.74	3231.43	−1947.62	−3615.25
$S(\theta_2 y)$	−1100.29	1037.59	3069.95	1363.18
$S(\theta_3 y)$	264.81	1226.46	557.25	−1506.94
$S(\theta_4 y)$	868.01	−2635.16	−5377.19	−2162.12
$S(\theta_5 y)$	524.42	−2307.24	−3083.17	78.36

Difference in yield of Garnet and Reward

$\gamma_0 = .0090922$ $\qquad \gamma_3 = .0143016$

$\gamma_1 = -.0080484$ $\qquad \gamma_4 = .0174913$

$\gamma_2 = .0001092$ $\qquad \gamma_5 = .0105813$

Difference in yield of Garnet and Marquis

$\gamma_0 = -.0151360$ $\qquad \gamma_3 = -.0254974$

$\gamma_1 = .0221254$ $\qquad \gamma_4 = -.0449486$

$\gamma_2 = -.0073164$ $\qquad \gamma_5 = -.0407105$

Difference in yield of Garnet and Red Fife

$\gamma_0 = -.0048636$ $\qquad \gamma_3 = -.0114844$

$\gamma_1 = -.0061265$ $\qquad \gamma_4 = -.0567342$

$\gamma_2 = .0180241$ $\qquad \gamma_5 = -.0612737$

Difference in yield of Marquis and Red Fife

$\gamma_0 = .0133333$ $\qquad \gamma_3 = -.0079812$

$\gamma_1 = -.0079323$ $\qquad \gamma_4 = -.0213125$

$\gamma_2 = .0096345$ $\qquad \gamma_5 = -.0052979$

The proportion of the yield variance accounted for by the respective regression formulas is shown in Table XXI. This is uniformly low except in the case of the difference Garnet–Reward. Even this, however, by no means attains the level of significance. It cannot be said, therefore, that these data reveal any consistent association between temperature conditions during the growing season and varietal differences in yield.

TABLE XXI

DISTRIBUTION OF SEASONAL VARIANCE OF VARIETAL YIELD DIFFERENCES

Variance	Garnet–Reward	Garnet–Marquis	Garnet–Red Fife	Marquis–Red Fife
Sum of squares:				
Due to rainfall regression (6 degrees of freedom)	65.14	179.69	540.58	257.71
Due to temperature regression (6 degrees of freedom)	52.09	305.18	557.24	145.78
Deviations from regression (30 degrees of freedom)	466.09	1416.61	1546.81	739.16
Total (42 degrees of freedom)	583.32	1901.48	2644.63	1142.65
Mean square:				
Due to rainfall regression	10.86	29.95	90.10	42.95
Due to temperature regression	8.68	50.86	92.87	24.30
Deviations from regression	15.54	47.22	51.56	24.64

Temperature and Yield of Wheat from Summerfallowed and Stubble Land

The sums of products due to the seasonal covariance of the rainfall and temperature coefficients will be found in Table XXII, and the regression of the temperature coefficients on the rainfall coefficients, calculated from these sums of products by means of the c values of Table X, in Table XXIII. Tables XXIV and XXV show the sums of products of yield and temperature coefficients, and of the yield and temperature residuals, respectively.

TABLE XXII

SUMS OF PRODUCTS OF SEASONAL DEVIATIONS OF RAINFALL AND TEMPERATURE COEFFICIENTS (SERIES III)

	θ_0	θ_1	θ_2	θ_3	θ_4	θ_5
ρ_0	441.8201	−788.6703	−701.4976	195.1780	144.0828	184.3352
ρ_1	−422.1688	40.4009	233.1662	30.9984	−217.2446	56.8434
ρ_2	−161.5960	315.0244	276.9642	−309.6751	527.6116	80.6117
ρ_3	36.2710	−444.0487	39.4245	−235.5485	86.5130	51.6520
ρ_4	8.5924	−289.1353	−429.1750	357.2893	−553.9455	−156.0151
ρ_5	9.1924	43.2061	181.6013	−273.6773	590.4029	−302.0484

TABLE XXIII

REGRESSION OF TEMPERATURE COEFFICIENTS ON RAINFALL COEFFICIENTS (SERIES III)

	θ_0	θ_1	θ_2	θ_3	θ_4	θ_5
ρ_0	49.06755	−60.87200	−88.79662	32.08566	17.10857	37.74296
ρ_1	−62.80296	−17.41988	51.51523	−15.02830	−31.86609	7.20007
ρ_2	16.13444	12.53382	−41.02141	1.51637	58.73163	20.87404
ρ_3	−36.06837	−42.29236	52.34389	−36.77078	−20.41775	−5.30237
ρ_4	6.70989	−10.06720	−40.68117	25.53857	−22.64785	−4.35172
ρ_5	−19.12481	30.82053	57.72701	−43.54940	55.66114	−42.50655

TABLE XXIV

SUMS OF PRODUCTS OF YIELD AND TEMPERATURE COEFFICIENTS

	Stubble	Summer-fallow	Difference, summer-fallow − stubble
$S(\theta_0 y)$	11092.36	12419.15	1326.79
$S(\theta_1 y)$	−15506.33	−17499.16	−1992.83
$S(\theta_2 y)$	−7682.48	−7037.33	645.15
$S(\theta_3 y)$	−1904.54	−305.71	1598.83
$S(\theta_4 y)$	1635.25	−1633.74	−3268.99
$S(\theta_5 y)$	990.28	2880.99	1890.71

TABLE XXV

SUMS OF PRODUCTS OF YIELD AND TEMPERATURE RESIDUALS

	Stubble	Summer-fallow	Difference, summer-fallow − stubble
$S(\theta_0 y)$	5683.49	7263.69	1580.20
$S(\theta_1 y)$	−7583.85	−8737.51	−1153.66
$S(\theta_2 y)$	−828.16	685.33	1513.49
$S(\theta_3 y)$	−3559.59	−3096.87	462.72
$S(\theta_4 y)$	80.28	−1611.21	−1691.49
$S(\theta_5 y)$	−645.60	1362.21	2007.81

The sums of squares and products of the seasonal deviations of the temperature coefficients are:

	θ_0	θ_1	θ_2	θ_3	θ_4	θ_5
θ_0	313126.8	−209260.8	−82955.1	−23973.3	26458.9	4804.4
θ_1	−209260.8	346463.7	51448.4	81359.4	6196.9	−8256.4
θ_2	−82955.1	51448.4	209450.0	−98929.8	1136.6	−24013.9
θ_3	−23973.3	81359.4	−98929.8	272087.4	−152747.6	28170.2
θ_4	26458.9	6196.9	1136.6	−152747.6	245352.8	−44700.7
θ_5	4804.4	−8256.4	−24013.9	28170.2	−44700.7	115230.8

and the corresponding values required in the estimation of the regression of the yield residuals on the temperature residuals are:

	θ_0	θ_1	θ_2	θ_3	θ_4	θ_5
θ_0	268968.0	−186357.8	−30584.6	−42734.2	15361.4	−4838.0
θ_1	−186357.8	272188.8	1086.9	99731.5	−15544.2	12867.4
θ_2	−30584.6	1086.9	116502.9	−53235.8	−14380.3	1118.8
θ_3	−42734.2	99731.5	−53235.8	237056.0	−118395.6	15717.4
θ_4	15361.4	−15544.2	−14380.3	−118395.6	161335.8	36444.0
θ_5	−4838.0	12867.4	1118.8	15717.4	36444.0	92937.4

resulting in the values of γ listed below:

Yield from stubble land:

$\gamma_0 = .0039530$ $\quad\quad$ $\gamma_3 = -.0214618$

$\gamma_1 = -.0187830$ $\quad\quad$ $\gamma_4 = -.0208558$

$\gamma_2 = -.0183526$ $\quad\quad$ $\gamma_5 = .0078885$

Yield from summerfallowed land:

$\gamma_0 = .0129911$ $\quad\quad$ $\gamma_3 = -.0338827$

$\gamma_1 = -.0154951$ $\quad\quad$ $\gamma_4 = -.0482360$

$\gamma_2 = -.0123744$ $\quad\quad$ $\gamma_5 = .0422734$

Difference in yield from summerfallowed and stubble land:

$\gamma_0 = .0090381$ $\quad\quad$ $\gamma_3 = -.0124289$

$\gamma_1 = .0032879$ $\quad\quad$ $\gamma_4 = -.0273802$

$\gamma_2 = .0059782$ $\quad\quad$ $\gamma_5 = .0343849$

The limited extent to which the residual variance of either the observed yields or the difference in yield between the stubble and fallow plots can be represented by means of these regression coefficients is shown in Table XXVI. However, there is a significant correlation between temperature conditions and the difference in yield of the Marquis plot from the variety series and the fallow plot of the present series. It is possible, therefore, that the relations illustrated in Figs. 5 and 6, are not wholly chance effects.

TABLE XXVI

DISTRIBUTION OF SEASONAL VARIANCE OF YIELD FROM SUMMERFALLOWED AND STUBBLE LAND

Variance	Stubble yields	Summer-fallow yields	Difference in yield
Sum of squares:			
Due to rainfall regression (6 degrees of freedom)	1374.22	1692.98	127.71
Due to temperature regression (6 degrees of freedom)	249.74	461.51	129.14
Deviations from regression (30 degrees of freedom)	2081.53	2036.78	1566.65
Total (42 degrees of freedom)	3705.49	4191.27	1694.36
Mean square:			
Due to rainfall regression	229.04	282.16	21.28
Due to temperature regression	41.62	76.92	21.52
Deviations from regression	69.38	67.89	47.92

Discussion of Results

As the significance of the estimated temperature effects is not definitely established, and as the coefficients γ are, owing to the high residual variance, subject to relatively large errors, an extensive discussion of these results would be unprofitable.

The extent to which the observed yields are affected by temperature conditions other than those associated with rainfall or the absence of rainfall may be judged from Tables XVI, XXI and XXVI. It has been suggested by Blair (5) that a considerable part of the apparent effect of either precipitation or temperature upon yield is really due to the accompanying effect of the other. Contrasting the numerical values of α and γ, however, and remembering that the standard deviation of the temperature coefficients

θ is from 110 to 150 times that of the corresponding rainfall coefficients ρ, it is seen that the total effect on yield of temperature variations (as portrayed by the available measurements) is to be judged secondary to that of fluctuations in rainfall. The value of R, the coefficient of multiple correlation between yield, and the rainfall and temperature coefficients, is 0.58 for the Marquis plots of the varietal test series and 0.66 and 0.72 for the stubble and fallow plots of the grain rotation respectively.

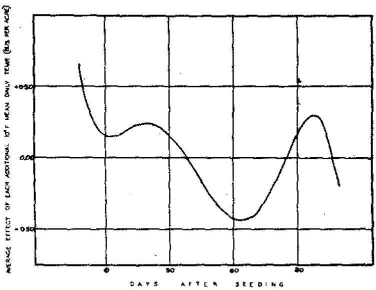

FIG. 5. *Average effect of temperature (eliminating influence of associated rainfall) on yield of Marquis wheat (variety test plot data).*

The curves of Figs. 5 and 6 agree in suggesting that above-average temperatures are beneficial at the commencement of the growing season, detrimental during midsummer, and again desirable during the period of maturation; these various phases succeeding each other at somewhat shorter intervals of time on the less fertile soil of the grain rotation (Fig. 6) than on the more fertile soil of the variety plots (Fig. 5). Such effects are in reasonable accordance with expectation. The reduction in yield accompanying high temperatures still later in the season might be due partly to the acceleration of respiration in the ripe grain, with increased losses of carbohydrate material, and partly to reduction of the moisture content of the grain at the time of threshing and weighing.

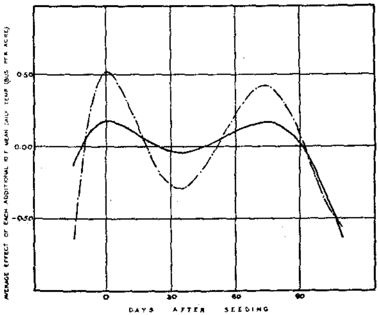

FIG. 6. *Average effect of temperature (eliminating influence of associated rainfall) on yield of wheat from stubble plot (solid line) and summerfallow plot (broken line) of grain rotation.*

Reference has already been made to the greater moisture-holding capacity of the variety test plot soils, and the higher specific heat of moist soil will of course lead to slower warming in the spring. Above-average air temperatures before seeding may be desirable therefore in order to raise the temperature of these relatively moist soils to levels more favorable to the germination of the seed and the early growth of the plant. This consideration will apply

with diminished force in the case of the grain-rotation plots; and on the other hand, owing to its lower fibre content, their soil is liable to blow in the spring. The annual reports of the superintendents of the experimental stations contain frequent references to such occurrences, which must be aggravated by the drying out of the surface layer, a process which will undoubtedly proceed more rapidly at higher temperatures. Even in these circumstances, however, additional warmth during the period of germination and early growth appears advantageous.

As the temperature conditions in the spring vary more than do those of the succeeding summer months (Fig. 7) it seems likely that some of the most

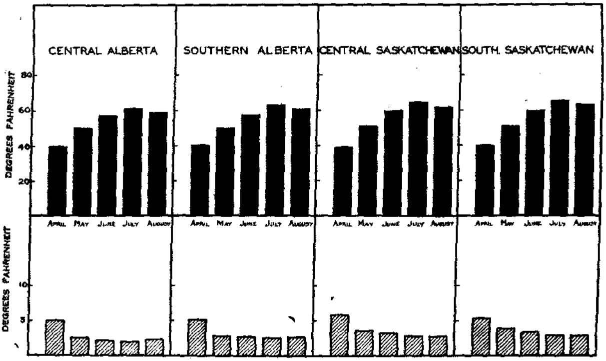

FIG. 7. *Upper portion: Average mean monthly temperature (degrees Fahrenheit) recorded at meteorological stations in central and southern Alberta and Saskatchewan. Lower portion: Annual variation (standard deviation in degrees Fahrenheit) in mean monthly temperature.*

pronounced effects of temperature upon yield may be exerted at this time. At all stations the month of July is characterized by the maximum mean temperature. That both the average values and the annual variation (standard deviation) of the corresponding temperatures are in general higher at the southern than at the northern, and at the Saskatchewan than at the Alberta stations, is also to be observed from Fig. 7.

7. General Conclusions

Cole and Matthews (9), studying the use of water by spring wheat on the Great Plains, found a correlation of 0.76 between the total quantity of water removed from the soil and the yield of grain. The supply of moisture upon which the crop may draw is derived from the amount available in the soil at the time growth begins, supplemented by the rain falling during the growing period. The former they found quite inadequate to carry the crop

to maturity, and under western conditions both combined are seldom in excess of the optimum requirements of the crop. In these circumstances the yield secured might certainly be expected to be in some degree dependent on summer rainfall; and the results of the present investigation, as well as the significant correlation between yield and seasonal precipitation found by the above authors, indicate that this is the case. The nature of the relation seems to be influenced by the soil conditions, and even under similar soil conditions is doubtless affected by the amount of moisture originally present. If this is so, the various curves of the preceding sections may be expected to indicate the average relation in the case of the soils, stations and seasons studied.

In the same way the practical importance of any "critical periods" in plant development will depend not on the absolute moisture and temperature requirements of the plant, but on the deviation of these requirements from the environmental conditions experienced at that time. Disparities between the rainfall response curves of different plots, as in Figs. 1 and 3, are not therefore, necessarily incompatible with the existence of relatively "critical" periods of plant growth in the sense referred to in Section 1; but they do suggest that the extent to which yields are actually limited by the rainfall during such periods may be subject to considerable modification by soil factors. Temperature conditions affect the plant more directly, and this circumstance perhaps accounts for the greater degree of similarity between the effects illustrated in Figs. 5 and 6.

Whatever may be the expectations on physiological grounds, the results of this study indicate that in the region to which they apply, the influence of weather conditions on wheat yield is not largely exerted in a few relatively short periods of time, but extends at least throughout the growing season. This conclusion is of importance in the formulation of prediction equations designed to forecast commercial yields from the known weather conditions. The limited effect upon yield of temperature conditions other than those associated with rainfall also has an obvious bearing in this connection, though in view of the tendency of cultivators to commence seeding operations as early as possible each spring, the observation that low temperatures during the initial stages of growth are associated with reduced yields may not be without practical significance.

It might be thought that the moderate degree of correlation between weather conditions and the plot yields studied, renders unlikely the successful forecasting of commercial production; but this is not necessarily so. The crop returns considered, being obtained from a different small area of land at each station each year, were undoubtedly affected by soil differences. If, however, the total production of a district is considered, soil differences above and below the average may be expected to a considerable extent to offset each other, resulting in a greater relative importance of crop variation due to seasonal weather conditions.

The results of Section 6, indicating that the yield secured is influenced less by temperature conditions than by rainfall during the growing season, are of interest in view of Chilcott's conclusion (8) that seasonal precipitation is not the dominant factor determining crop yields in the Great Plains area. Whilst the "inhibitory factors" such as hot winds, diseases, and insects, to which this author rightly directs attention are undoubtedly of great importance in some seasons, it seems probable that the statistical method employed by him, namely the calculation of the ratio of the yield of grain to the seasonal precipitation, as well as the fact that the total precipitation only, regardless of its distribution, was considered, has led to some under-estimation of the actual association between yield and rainfall. It is obvious, however, that the yields secured are markedly influenced by factors other than rainfall and temperature during the growing season, and the amount of precipitation during the autumn, winter and spring months prior to sowing is being studied in this connection.

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SOME FACTORS IN THE DIFFERENT CHROMOSOME SETS OF COMMON WHEAT[1]

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Abstract

In crosses between 21-chromosome (*vulgare*) and 14-chromosome (emmer) wheats many hybrid lines which had only 14 pairs of chromosomes were identified and were then studied from the standpoint of their emmer and *vulgare* characters. Any *vulgare* character which appears in such lines must be determined by genes in the primary set of 14 chromosomes which mate in F_1 with those of emmer. *Vulgare* characters not appearing in such lines are determined by genes in the secondary set of 7 which remain unmated in F_1. In this way it has been determined that 7 of the 19 characters studied in *durum* crosses and 9 of the 21 studied in *dicoccum* crosses are determined by genes in the primary set of 14 (for list see Table II). For several other *vulgare* characters it has been shown that necessary genes are in both the primary 14 and the secondary 7 (Table III). There is little to indicate that genes determining important species-distinguishing characters are concentrated in the secondary set of 7, as is implied in the theory that *vulgare* wheat has resulted from the crossing of an emmer with *Aegilops* and that the set of 7 is derived from *Aegilops* and homologous with a set in that genus. Evidence in regard to the mode of inheritance of certain characters is given.

Introduction

All of the stable lines which result from crossing common wheats with emmer types have the parental chromosome numbers ($n = 21$ or 14). The offspring of hybrids with intermediate numbers tend to revert to those of the parents (1, 2, 4) and eventually the reversion becomes complete in all lines. The inheritance of any character of common wheat will therefore depend, among other things, on whether it is determined by genes in the two sets of 7 which mate with those of emmer (hereinafter called the primary 14 or sets A and B), or by genes in the 7 which remain unmated in F_1 (hereinafter called secondary 7 or set C).

If a *vulgare* character appears in a hybrid segregate which has only 14 pairs of chromosomes, all the genes necessary for its production must be located in the primary 14 (A and B). A hybrid line with 14 pairs only cannot show a common wheat character whose genes are in the secondary 7 (C); nor can a line with 21 pairs show an emmer character whose *vulgare* counterpart is determined by genes in the secondary 7, unless the emmer character is epistatic to that of *vulgare*. The substitution of a particular emmer character for a *vulgare* one in a hybrid of the general *vulgare* type will therefore depend on whether the *vulgare* genes involved are in the primary 14 or in the secondary 7.

The purpose of this investigation has been to determine in which set of chromosomes (A and B on the one hand or C on the other) the genes for many characters are located. This has been done by determining whether the

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characters in question occur in 14-chromosome* hybrid lines or whether their emmer counterparts occur in 21-chromosome hybrid lines. The results obtained with the 14-chromosome lines are given in the following pages. The results with 21-chromosome lines which are more difficult to obtain and study in the necessary large numbers, are not as yet sufficiently extensive to report. Supporting evidence has been obtained by determining whether *vulgare* characters which are not found in 14-chromosome lines occur in plants which have 15 (14 bivalents and one of the C chromosomes).

This problem is given additional significance by the widely held view, originally suggested by Percival (3) on taxonomic grounds, that common wheat originated through the crossing of an emmer type with *Aegilops*, and that the characters which distinguish common wheat from emmers were contributed by that genus. This conception has been given a precise cytological significance by recent observations, which are taken to indicate that the secondary set of 7 chromosomes (C) present in *vulgare* wheat has come from *Aegilops* and therefore should carry the distinctively *vulgare* genes. The cytological observations on which this conclusion is based have been reviewed by Thompson (5).

Before cytological work had been done on *Aegilops* or its hybrids with wheat, Sax (4) had reached the conclusion from his studies on *vulgare*-emmer hybrids that the distinguishing characters of common wheat are determined by genes in the C chromosomes. He wrote "Apparently the 7 additional chromosomes of *vulgare* varieties determine the distinguishing characters of common wheats due to the reduplication of hereditary factors, or to specific factors in these chromosomes . . . . The cytological and genetic data would indicate that many of the desirable characteristic properties of the emmer and *vulgare* wheats cannot be combined in a homozygous condition."

In the present work no general attempt has been made to analyze the genetics of the different characters, to determine how many genes are involved or their mode of interaction. In most cases the results reported show only whether all the genes which are necessary for the production of the character in question are in the primary 14 chromosomes. In some cases it is shown that necessary genes are in both the primary 14 and the secondary 7.

Materials and Methods

In extensive earlier work on the cytology of wheat species crosses a large number of hybrid segregates had been identified as having only 14 bivalent chromosomes at the first meiotic division. Others had 14 bivalents and 1 univalent. Some of them were F_3 plants resulting from selfing F_1 and F_2 of *vulgare* × emmer. Many were the results of backcrossing F_1 to the emmer parent. Offspring of them were grown and studied both genetically and cytologically.

* *For the sake of clearness and ease in deciding which set is involved in each case haploid numbers are used throughout. "A 14-chromosome plant" means one which shows only 14 bivalent chromosomes at the first meiotic division and therefore has 2 of the primary sets of 14 or 28 somatic chromosomes. "A 15-chromosome plant" means one with 14 bivalents and 1 univalent, the latter belonging to the secondary 7.*

With respect to some of the distinguishing parental characters exact classification was difficult. Various intermediate conditions appeared. Several characters described as units in taxonomic work broke up into sub-characters in the hybrids. Entirely new conditions not found in either parent occurred. The exact condition was recorded as accurately as possible for each plant.

The chromosome number and mating at the first division were determined for several plants in each line by means of smear preparations. This not only checked the parental count but made it possible to state explicitly that a particular *vulgare* character had been found in a particular 14− (or 14+1) chromosome plant. Since all plants had 14 bivalent chromosomes plus 0 or 1 univalent it was only necessary to determine whether or not a univalent was present at the meiotic division. At least 20 pollen mother cells of each plant were examined.

The amount of material studied is shown in Table I. The total for 14-chromosome lines is 83 families and 757 individuals, and for 15-chromosome lines 34 families and 274 individuals. All lines are descended from different F_2 or backcross plants. The numbers should be large enough to make the results reliable. In addition six families including 38 individuals were examined from 14-chromosome backcrosses of *vulgare*×*persicum* F_1 to *persicum*.

TABLE I

NUMBER OF LINES AND PLANTS STUDIED (EACH LINE FROM A DIFFERENT F_2 OR BACKCROSS PLANT)

Chromosomes*	F_4 by selfing F_1, F_2, and F_3				Backcrosses			
	Durum crosses		*Dicoccum* crosses		With *durum*		With *dicoccum*	
	Lines	Plants	Lines	Plants	Lines	Plants	Lines	Plants
14 II	6	72	14	149	30	253	33	283
14 II + 1 I	2	27	8	77	13	99	11	72

* I = *univalent;* II = *bivalent.*

The parental varieties used were as follows: *vulgare*—Marquis; *durum*—Iumillo; *dicoccum*—Vernal; *persicum*—Black Persian.

The Characters Studied

There are very few characters which distinguish all varieties of *vulgare* from all varieties of *durum* or *dicoccum*. But there are several characters which are confined to one species although they are not found in all varieties of that species. And there are several characters which do not differ in kind as between two species but which are usually developed to a greater degree in one species than in the other. Moreover there are numerous characters which distinguish the great majority of individuals and forms of one species from those of the others and which may therefore be regarded as typical of the species in question. Watkins (7) has discussed at length the differences between and interrelationships of the species of *Triticum*.

The characters studied in the present work are in some degree species-distinguishing, although, as indicated above, few of them distinguish all forms of *vulgare* from all forms of *durum* or *dicoccum*. For example there is some overlapping in regard to the diameter of the stem but *vulgare* forms as a rule have thicker stems than *durum* or *dicoccum*. The absence of beards is a feature of many *vulgare* types (including the one used in this study), but not of all; beards are never absent from *durum* or *dicoccum* types. The descriptions which follow apply to the particular forms used in this study.

1. Diameter of stem—measured at 2 cm. below the spike; emmer (*durum* and *dicoccum;* hereafter designated "e") 1.1 to 1.5 mm.; *vulgare* (hereafter designated "v") 1.6 to 2.2 mm.
2. Cavity of stem—observed at 2 cm. below the spike; e solid, v hollow with thin walls.
3. Leaf hairs—of young leaves; *durum* none or very few, short and scattered; *dicoccum* very numerous, long, evenly distributed; *vulgare* long on tops of ridges, short on sides, sparse.
4. Collar—a collar-like structure surrounding the stem at the base of the spike; in e a complete circle; in v open at one side.
5. Density of spike—calculated by dividing the length of the spike in millimetres by the number of spikelets.
   The value so obtained is slightly greater than the average internode length. e 3 to 4; v 4.4 to 5.6.
6. Form of spike—width of one-ranked side divided by width of two-ranked side. In *dicoccum* 0.6 to 0.8; *durum* 0.7 to 1.0; *vulgare* 1.3 to 1.7.
7. Beards—e 8 to 12 cm.; v short tip awns.
8. Glume shape—empty glume, e, long, tapering to both ends; v, short, wide, blunt.
9. Glume cross section—e, V-shaped; v, U-shaped.
10. Glume tip—*durum* long, sharp; *dicoccum* short, pointed; *vulgare* short, blunt.
11. Glume shoulder—the portion at the side of the tip; e narrow, sloping; v wide, more horizontal.
12. Glume adherence—*dicoccum* tight, difficult to remove; *durum* and *vulgare* loose, easily removed.
13. Keel—ridge on back of empty glume; e prominent, sharp, extending full length of glume; v prominent above, weak below.
14. Keel-teeth—*durum* fine, hair-like, numerous from tip to near base; *dicoccum* few, short, blunt, near the tip; *vulgare* short, blunt, sparse, on upper half.
15. Shape of segment of rachis between successive spikelets—e slender, top twice as wide as base; v stout, top little wider than base, margin curved.
16. Rachis hairs—*durum*, a marginal tuft at the top of each segment tapering downwards; *dicoccum*, a central tuft between the glume bases; *vulgare*, a uniform fringe along the sides and across the top.

17. Rachis articulation—*dicoccum* fragile, the spike readily breaking into segments; *durum* and *vulgare* tough, remaining intact during threshing.
18. Seed shape—e long, narrow, tapering to ends; v short, stout, blunt.
19. Seed cross section—e somewhat triangular with ridge on back; shallow furrow; v rounded back and sides, deep furrow.
20. Seed end—embryo end; *durum* one projection; *dicoccum* a longitudinal furrow; vulgare two blunt projections.
21. Seed hairs—on stigma end; *durum* few, short, *dicoccum* many short; *vulgare* many long.

Experimental Results

A. *Vulgare* Characters in Segregates with 14 Pairs of Chromosomes

The characters of the 21-chromosome species (*vulgare*) which have been found in 14-chromosome hybrid lines are shown in Table II. In regard to leaf hairs only a single plant with 14 chromosomes in *durum* crosses and backcrosses was recorded as having the *vulgare* condition. The same is true with respect to the cross section of the glume. Since the possibility of single errors in the records cannot be entirely ruled out, it may be questionable whether the *vulgare* condition of these two characters has actually been found in 14-chromosome *durum* segregates. There is no doubt that they occur in 14-chromosome *dicoccum* segregates. In regard to all the other characters more than one family and several plants in a family showed the *vulgare* condition.

TABLE II

Vulgare characters found in 14-chromosome lines and number of lines in which they occurred

Character	F_4 of crosses with		F_2 of backcrosses with	
	durum	*dicoccum*	*durum*	*dicoccum*
Stem diameter	2	2	6	1
Leaf hairs	1	6	0	9
Beards	5	11	9	8
Glume section	1	2	0	2
Glume tip	3	4	9	7
Glume adherence	–	5	–	7
Keel	0	6	0	11
Keel teeth	0	5	0	7
Seed hairs	2	0	4	0
Seed end	2	8	2	5
Lines studied	6	14	30	33

It will be observed that seven (or possibly only five) of the 19 *vulgare* characters have been found in 14-chromosome *vulgare-durum* hybrids, and nine of the 21 *vulgare* characters in 14-chromosome *vulgare-dicoccum* hybrids. The total number of characters studied is different in the two crosses because in regard to two of them, glume adherence and rachis articulation, *vulgare* is similar to *durum* and quite different from *dicoccum*. The reason for the greater ease with which *vulgare* characters can be transferred to 14-chromosome plants in *vulgare-dicoccum* than in *vulgare-durum* crosses will be discussed later. But the evidence from both crosses shows that by no means all *vulgare* characters are determined by the secondary seven chromosomes.

Furthermore the *vulgare* characters which have been transferred include some important species-distinguishing characters such as the condition of the leaf hairs, cross section of the glume, diameter of stem, and the keel. But they also include some which do not distinguish all *vulgare* varieties from all emmer varieties, such as absence of beards, or blunt glume tip.

It is of interest to note that with respect to ten of the 21 characters studied in *vulgare-dicoccum*, the F_1 is intermediate; with respect to nine others the *vulgare* condition is partially or completely dominant; with respect to the remaining two it is recessive. Of the nine characters transferred four are intermediate in F_1, four are partially or completely dominant and one recessive. In *vulgare-durum* four of the five characters transferred are dominant in some degree, while one is intermediate in F_1.

In view of the large amount of sterility and chromosome irregularity in F_1 it is not considered advisable to draw any conclusions with respect to the mode of inheritance from the proportion of families in which a character appeared.

Some characters which have not been found in the fully developed *vulgare* condition in 14-chromosome plants, have been found in a near-*vulgare* condition. These are shown in Table III. Presumably important genes influencing them are located in the primary 14 chromosomes but for their complete expression genes located in the secondary 7 are necessary in addition. It may be, however, that all the genes necessary for the expression of some of them are in the primary 14 and that emmer genes which inhibit their full development have not been entirely eliminated from any of the plants studied.

TABLE III

CHARACTERS FOUND IN NEAR-*vulgare* CONDITION IN 14-CHROMOSOME LINES AND NUMBER OF FAMILIES IN WHICH THEY OCCURRED

Character	F_4 of crosses with		F_2 of backcrosses with	
	durum	*dicoccum*	*durum*	*dicoccum*
Spike density	–	–	6	3
Glume shape	2	3	3	2
Glume shoulder	2	4	7	3
Rachis hairs	1	1	2	3
Rachis segment	4	2	4	0
Rachis articulation	–	2	–	1
Seed shape	1	6	3	2
Lines studied	6	14	30	33

Most of those characters which have been found in the near-*vulgare* condition in 14-chromosome plants have been found in the completely *vulgare* condition in plants which have a single C chromosome in addition to the 14 pairs. This is shown in Table IV. It will be observed that the characters listed in this table are almost the same as those in Table III. Presumably the extra chromosome from the secondary 7 carries the gene which is necessary, in addition to those in the primary 14, for the full expression of the *vulgare* condition. (The same *vulgare* characters which are found in plants which

have no univalent chromosomes have, of course, been found in those plants which have one univalent in addition, but they have not been shown in the table.)

New characters found in Table IV may of course be due entirely to genes in the additional chromosomes. Since there are seven extra chromosomes and since the number of families with an extra chromosome was small, it is probable that further work on such lines will add more characters to this category. It should add all those *vulgare* characters which are determined by a single kind of gene in the secondary 7, except those for which the gene must be represented twice as in pure *vulgare*.

TABLE IV

CHARACTERS FOUND IN *vulgare* CONDITION IN PLANTS WITH 14 BIVALENT AND 1 UNIVALENT CHROMOSOMES

Character	F_4 of crosses with		F_2 of backcrosses with	
	durum	*dicoccum*	*durum*	*dicoccum*
Spike form	1	–	2	1
Spike density	–	1	2	2
Collar	1	2	3	2
Glume shape	–	2	–	–
Glume shoulder	–	3	1	3
Rachis hairs	1	1	1	–
Rachis segment	–	2	–	–
Rachis articulation	–	2	–	–
Seed shape	–	1	1	–
Lines studied	2	8	13	11

In addition to the *durum* and *dicoccum* crosses which have just been described, the progenies of six 14-chromosome plants resulting from backcrossing *vulgare*×*persicum* F_1 to *persicum* were studied. *T. persicum* differs from the variety of *vulgare* which was used, in having black pubescent glumes, lighter seed color and rugose caryopsis, in addition to many of the characters which distinguish *durum* and *dicoccum* from *vulgare*. The *vulgare* seed and glume color and absence of pubescence were found among the six families. A near-*vulgare* condition of the surface of the caryopsis was also found.

B. ADDITIONAL RESULTS AND THE MODE OF INHERITANCE OF CERTAIN CHARACTERS

In regard to certain characters additional evidence was obtained which cannot be given in tabular form. Some of this evidence bears on the mode of inheritance of certain characters. Although a complete analysis of the mode of inheritance was not attempted, and would be very difficult owing to sterility and chromosome irregularity in F_1 certain conclusions may be pointed out.

1. Cavity of Stem

No 14-chromosome hybrid has been obtained with the hollow, thin-walled stem characteristic of *vulgare*. Many have been obtained with small cavities but they all had thick walls. It would appear, therefore, that a factor for

hollow stems is present in the primary 14 chromosomes of *vulgare* and that in order to produce the large cavity with thin walls at least one additional factor located in the secondary 7 must be present.

2. Leaf Hairs

T. vulgare has long hairs on the crests of the ridges of the young leaves and short ones on the sides of the ridges; in the variety used, both kinds are sparse. *T. dicoccum* has numerous long hairs uniformly distributed, and *durum* has very few short ones or none at all. The *vulgare* condition was transferred without difficulty to 14-chromosome *dicoccum* hybrids and is therefore determined by genes in the primary 14 chromosomes. Consequently it should be transferred to 14-chromosome *durum* hybrids. But this occurred in only one case. It may be that *durum* genes inhibit the *vulgare* condition and only one plant was obtained which possessed the necessary *vulgare* chromosomes and not the *durum* chromosomes which carry the inhibiting genes. It is of interest in this connection that in *vulgare* × *durum* F_1 the *durum* condition is nearly dominant, the hairs being few and short, and that in *vulgare* × *dicoccum* the hairs are numerous as in *dicoccum*, mostly rather long, with some still longer on the crests of the ridges as in *vulgare*.

Possibly the fact that the *vulgare* condition of the leaf hairs is readily transferred to *dicoccum* but not to *durum* hybrids may be due to the absence of genes for long hairs in *durum* and the primary 14 of *vulgare* and their presence in *dicoccum* and the secondary 7 of *vulgare*. In that case there would be no genes for long hairs in 14-chromosome *durum* hybrids; the long hairs of *dicoccum* hybrids would be due to genes from *dicoccum*, and the difference in length between the top and the side of ridges would be due to genes in the primary 14 of *vulgare*.

According to Percival (3) the arrangement, number, and kind of hairs in the young leaves of wheat are among the most constant species-distinguishing characters. In the hybrids these are very variable. It appears that different genes affect the characters since various combinations of the numbers, lengths, and arrangements of the parental species were observed. But all the genes necessary to produce the *vulgare* condition are apparently in the primary 14 chromosomes.

3. Density of Spike

In several 14-chromosome lines the spikes were almost, but not quite, as lax as in pure *vulgare*. Apparently there is a spike-lengthening gene (or genes) in the primary 14 chromosomes but an additional gene (or genes) is required from the C chromosomes in order to produce the full *vulgare* laxity. There is a similar situation with respect to several other characters. It may be mentioned also that some of the backcrosses to *vulgare* (not reported in this paper) had even laxer spikes than *vulgare*. This indicates either that *durum*, though very dense, contains spike-lengthening factors not present in *vulgare*, or that *vulgare* contains spike-shortening factors which have been eliminated in the hybrids.

4. Glume Tip

The short blunt tip on the empty glume which is characteristic of *vulgare*, appeared in many 14-chromosome hybrids. In addition several lines had a much longer, sharper tip even than *durum;* it was in fact a short awn. One of the parents must carry, in the primary 14 chromosomes, genes for a short awn on the empty glume and also genes which suppress the awn. In some hybrids the awn-producing gene is present without the suppressor.

5. Keel

The weakly developed keel characteristic of *vulgare* was present in many 14-chromosome *vulgare*×*dicoccum* hybrids. Some had an even weaker keel than *vulgare.* But the *vulgare* condition was not found in *durum* hybrids. The nearest approach to it was a low but distinct keel extending the full length of the glume. It appears that factors are present in *durum* and not in *dicoccum* which promote keel development and that these have not been eliminated in any of the *durum* hybrids studied. There is a similar difference between *durum* and *dicoccum* crosses in the difficulty of transferring the *vulgare* condition of keel teeth and of leaf hairs. With respect to the latter two characters, however, *dicoccum* resembles *vulgare* much more closely than does *durum.* The fact that three *vulgare* characters can be readily transferred in *dicoccum* but not in *durum* crosses therefore has no general significance. One character, seed hairs, was transferred in *durum* crosses but not in *dicoccum* crosses.

In a cross between *vulgare* and *turgidum* Watkins (6) found plants without keels or with keels even less developed than in *vulgare.* His interpretation is that *turgidum* has the factors KK for the prominent keel and that *vulgare* has kk for the absence of keel in the primary 14 and K_1K_1 for weak keel in the secondary 7. Keel-less extracted hybrids are then kk; 21-chromosome plants of the genotype kkK_1K_1 have the *vulgare* keel, and those of the genotype KKK_1K_1 have an even more strongly developed keel than *turgidum.* The facts mentioned with respect to the *vulgare-dicoccum* cross support this interpretation, and the inheritance of other characters, such as glume tip, may be interpreted in a similar way.

6. Shape of Rachis Segment

In *durum* and *dicoccum* the segment of the rachis between successive spikelets is narrow, slender and somewhat triangular, the top being nearly twice as wide as the base; in *vulgare* it is thick and stout, with a characteristic marginal curve and with the top little wider than the base. Such a variety of conditions was found in the hybrids as to indicate that several gene differences are involved. It may be that all the genes necessary to produce the *vulgare* condition are in the primary 14 chromosomes but not all have been obtained in one plant. Since certain elements of the *vulgare* condition are readily obtained in 14-chromosome plants it appears that some of the necessary genes are in the primary 14.

7. Rachis Hairs

Durum has a marginal tuft of hairs at the top of each segment, decreasing in length in a downward direction; *dicoccum* has a central tuft between the bases of the glumes; *vulgare* has a uniform fringe of hairs along the full length of the margin and across the top. Various combinations of these conditions were noted in the hybrids. For example some had tufts in both the *durum* and *dicoccum* positions; some had a near-*vulgare* fringe with a *dicoccum* tuft some had no hairs at all. The character is apparently influenced by several genes and some of these are in the secondary 7 chromosomes.

Discussion

It has been pointed out that if a *vulgare* character occurs in a hybrid segregate which has only 14 bivalent chromosomes, all the genes necessary for its expression must be in the primary 14 chromosomes which mate with those of emmer. Five (or possible seven) of the characters studied fall in this category in *durum* crosses, and nine in *dicoccum* crosses. If such a character appears in any considerable proportion of 14-chromosome plants, genes in only one or a few chromosomes must be sufficient for its expression. Several characters appear to be of this kind.

If a *vulgare* character is partly but never fully expressed in 14-chromosome segregates two explanations are possible: (*i*) Some of the *vulgare* genes influencing the character are in the primary 14 and some in the secondary 7 chromosomes, or (*ii*) all are in the primary 14 but the presence of emmer genes, whether allelomorphic or not, inhibits the full development of the character. The latter intrepretation assumes that in all the plants examined the emmer chromosomes carrying the modifying genes are still present. The question then is whether the number of plants examined is large enough to include some which have only *vulgare* chromosomes carrying the genes influencing the character. The number examined in the present study appears to be large enough, unless the character is influenced by genes in several chromosomes. Therefore the first interpretation appears in general to be correct—the *vulgare* characters in question require for their full development, genes in both the primary 14 and the secondary 7.

This conclusion is supported by the fact that nearly all the characters in this category appear in the full *vulgare* condition among plants which have an extra C chromosome.

It is clear from the results that the primary 14 chromosomes carry all the genes necessary for the production of several *vulgare* characters and part of those necessary for the production of others. There is little to indicate that the important species-distinguishing genes are concentrated in one set of chromosomes rather than the other. This conclusion is not in agreement with that which was reached when these interspecific crosses were first studied (4). It was thought that the specifically *vulgare* genes are concentrated in the secondary 7 chromosomes and that it would therefore be impossible to obtain 21-chromosome plants with emmer characters.

Some of the conclusions reached in this work are supported by the preliminary study of the extracted hybrids which have 21 chromosomes. These plants show the emmer counterparts of some of the *vulgare* characters which have been found in 14-chromosome segregates. The presence of the secondary 7 chromosomes has not caused the development of the *vulgare* characters in question.

According to a theory which has received wide recognition, *vulgare* wheat originated through the crossing of a member of the genus *Aegilops* with an emmer wheat. *Aegilops* contributed the secondary set of 7 chromosomes which are still homologous with a set in *Aegilops*. It also contributed (in those chromosomes) the genes for the distinctively *vulgare* characters. If that theory is correct the genes must have been extensively shifted into the other sets by translocations. But there is no evidence of such translocations in the numerous reports of the behavior of the chromosomes at meiosis in hybrids.

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FRACTIONATION OF GLUTEN DISPERSED IN SODIUM SALICYLATE SOLUTION[1]

By A. G. McCalla[2] and R. C. Rose[2]

Abstract

The solubility of gluten in sodium salicylate solution increases regularly with increasing concentrations of the solvent. When gluten is completely dispersed and then precipitated by the addition of magnesium sulphate, the relation between the amount of gluten nitrogen precipitated and the concentration of magnesium sulphate is also regular. Successive fractions of the precipitated gluten protein (except the last 10-15%) contained progressively more amide and less arginine nitrogen. None of the fractions were similar to gluten, but when they were redispersed, combined and reprecipitated as a whole, a gluten was obtained. The most soluble 10-15% of the gluten protein is probably distinct, but the remainder is a single protein complex which can be progressively fractionated.

Introduction

Recently the view that gluten is composed of two distinct proteins, glutenin and gliadin, has been seriously challenged. These proteins, in common with most others, are defined chiefly on the basis of their solubilities, and there is considerable evidence that such definitions are only arbitrary. The fraction of gluten which is soluble in 70% alcohol has been called gliadin, and the remainder, which is soluble in dilute acid or alkali, glutenin.

Gortner, Hoffman and Sinclair (8), however, have shown that practically any amount from 15 to 60% of the protein in flour can be extracted by choosing suitable salt solutions. Doubtless for each salt solution there is a concentration which extracts the maximum amount of protein, and a further increase in concentration either does not affect, or else decreases, the amount extracted. Recently Cook and Rose (4) have shown that a sodium salicylate solution of sufficient concentration will disperse practically all of the protein in gluten. It appears that solubility in the salt solutions used by Gortner, Hoffman and Sinclair, or even in sodium salicylate solution, might be used in defining gluten proteins, with as much reason as solubility in alcohol, acid and alkali.

There is also other evidence that the definitions of glutenin and gliadin are only arbitrary. Thus Herd (9) has shown that the amount of gliadin obtained from samples of flour depends on the method of extraction, and believes that so-called "gliadin" is not an individual protein. Cook (2) fractionally precipitated gluten from a dispersion in 30% urea solution and concluded that, on the basis of solubility, there was no clear-cut distinction between glutenin and gliadin.

Chemical analyses have shown that so-called "glutenin" and "gliadin" differ in their amide and arginine content. Different workers have obtained gliadin samples of approximately constant amide and arginine nitrogen content and so, from the standpoint of consistent chemical composition, gliadin may

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lay some claim to individuality. This, however, is not true of so-called "glutenin", as different workers have obtained widely divergent results (3). Sandstedt and Blish (14) fractionated the gluten protein by precipitation from dispersions in acetic acid, and found that the fractions produced differed gradually in chemical composition. They suggested that gluten is composed of three main proteins, the third being intermediate between glutenin and gliadin in its properties. Cook's results (2), however, show that the relation between solubility and arginine content of fractions was essentially linear.

In order to fractionate the gluten protein it is necessary to have a suitable agent which will disperse the gluten completely. Ideally, the reagent would be such that the protein could be recovered without the loss of any of its original properties. The known solvents are alkali, acid, urea, and sodium salicylate solutions. Sandstedt and Blish (14) showed that dilute acetic acid is preferable to dilute alkali, and the recent work of Cook and Rose (5, 6, 13) showed that the neutral solvents are superior to either alkali or acid.

Sodium salicylate was chosen for use in the present study since the use of urea involves the laborious procedure of removing the urea nitrogen.

Materials

Four flours were used in this study, namely: a commercial pastry flour, a low protein flour milled from Marquis wheat, a medium protein flour milled from a Reward wheat and a high protein flour milled from another Marquis wheat. These are designated respectively as Flours 1, 2, 3 and 4. The last three were experimentally milled and free from bleaching agents and chemical improvers. All but Flour 1 were of high baking quality. Analytical data concerning these flours are given in Table I. The methods used in determining these values are given in the following sections.

TABLE I

NITROGEN IN FLOUR AND WASHED GLUTEN

Flour	Total nitrogen in flour (dry basis), %	Nitrogen in washed gluten		
		Total, as percentage of total nitrogen in flour	Amide, as percentage of total in washed gluten	Arginine, as percentage of total in washed gluten
1	1.93	82.6	20.6	9.8
2	2.11	87.6	21.0	9.6
3	3.08	89.9	21.9	8.3
4	3.52	90.5	22.1	7.6

Methods

Washed Gluten

All glutens were washed from the flour, using the phosphate buffer solution of pH 6.8 recommended by Dill and Alsberg (7). The amount of flour weighed out depended on the experiment for which the gluten was used, and the rate of flow of the washing solution and the time of washing were varied according to the sample size.

traction of Gluten

Concentrations of sodium salicylate varying from 0 to 10% were employed, d the method was essentially that of Cook and Rose (4) in which the gluten shed from 5 gm. of flour was shaken at frequent intervals for six hours with) ml. of solvent. Cook and Rose found that increasing the amount of vent, or the time of extraction, had little effect on the amount dispersed, d although this was verified with the higher concentrations of sodium salilate as used by Cook and Rose, repeated extraction with the lower concentitions did slightly increase the amount extracted. A six hour extraction riod, however, was used with all solutions. When extraction was carried t with water it was first saturated with toluene to limit bacteriological tion. In the first experiments the extract was filtered through glass wool centrifuging left floating particles, but in subsequent experiments the foam oduced by shaking was broken by the addition of caprylic alcohol and the tract centrifuged. The results obtained by the two methods were slightly fferent as shown later.

reparation of Dispersions

Dispersion was accomplished after the method of Rose and Cook (13) by lacing the wet gluten in 10% solution salicylate solution and shaking vigorusly at frequent intervals. Sufficient wet gluten was used to give a conentration of approximately 5 mg. of nitrogen per ml. At the end of five ours when dispersion was complete the dispersion was placed in the refrigertor at 1° C. and left overnight. The following morning it was centrifuged t approximately 2500 r.p.m. for 20 min. and the liquid decanted into a olumetric flask of appropriate size, and made up to volume.

recipitation of Dispersed Protein

The protein was fractionally precipitated by the successive addition of alculated volumes of a magnesium sulphate solution, saturated at 20° C. An ttempt was made to prepare a saturated magnesium sulphate solution in 0% sodium salicylate but this was impossible, owing probably to the formtion of magnesium salicylate. When, however, equal volumes of saturated nagnesium sulphate and 10% sodium salicylate were mixed, no precipitate ormed even when the solution was stored at 1° C. for several days. After he addition of the salt the dispersion was mixed thoroughly by shaking, and tored at 1° C. for approximately 22 hr. It was then centrifuged at about 2500 r.p.m. for 20 min., the liquid decanted and the precipitate washed once with a sodium salicylate–magnesium sulphate solution of the same concentration as that in which precipitation occurred.

Total Nitrogen Determination

This determination was made by the Kjeldahl method, using mercuric oxide as the catalyst.

Nitrogen Distribution

This determination was restricted to amide and arginine analyses as these are the main groups that are known to differ quantitatively in so-called "glutenin" and "gliadin". The wet gluten or freshly precipitated protein was hydrolyzed for 24 hr. with 20% hydrochloric acid, the hydrolysate filtered and the residue washed. After making the filtrate up to a volume of 100 ml., aliquots were taken for total nitrogen and amide nitrogen determinations. The amide nitrogen was determined by the aeration method of Plimmer and Rosedale (11) except that magnesium oxide was used instead of calcium oxide. The arginine nitrogen was determined on the residue from the amide nitrogen determination, again using the method recommended by Plimmer and Rosedale (12).

Experiments

Extraction of Gluten

This experiment was conducted to determine the relation between the percentage of nitrogen extracted from washed gluten and the concentration of the sodium salicylate solution employed. The results are shown in Fig. 1. Since with the lower concentrations of sodium salicylate the gluten ball could not be broken up thoroughly, it is possible that the percentage extracted by these concentrations is too low. There is a difference between the results obtained by filtering the extract and by centrifuging it, and the percentage of nitrogen extracted by a given concentration of sodium salicylate obviously depends, to some extent, on the flour from which the gluten was washed. In all cases, however, the relation is regular, there being no deviations from the smooth curves that cannot be attributed to experimental error.

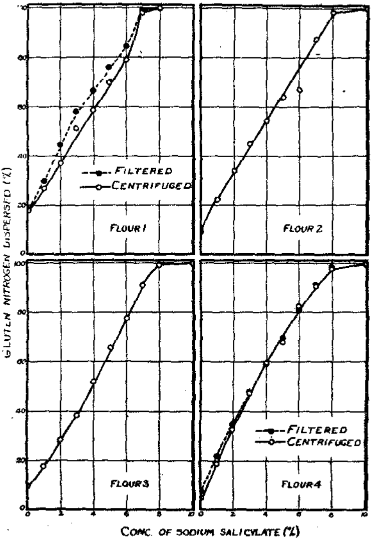

FIG. 1. *Extraction of gluten with sodium salicylate solutions.*

Precipitation of Dispersed Protein

The object of this experiment was to obtain the relation between the percentage

the total nitrogen precipitated and the concentration of the precipitat-g reagent. Only Flours 1 and 3 were used, as sufficient amounts of ıe other two were not available. Dispersions were prepared as already escribed and suitable aliquots pipetted into centrifuge tubes. The protein as then precipitated, washed, transferred to Kjeldahl flasks and the total itrogen determined directly.

The results are shown in Fig. 2. Since the magnesium sulphate solution 'as made up in water, its addition would decrease the concentration of sodium alicylate. This secondary factor probably affected the shape of the curves

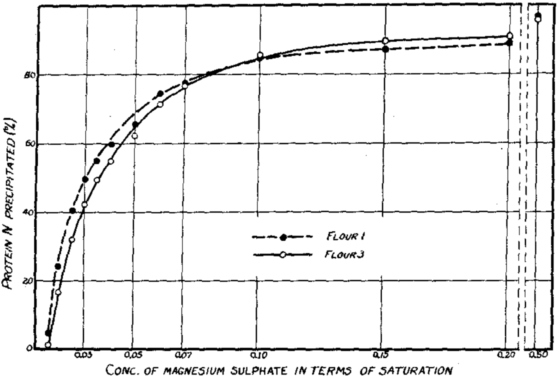

FIG. 2. *Precipitation of dispersed gluten with magnesium sulphate solution.*

in Fig. 2, but it could in no way affect the continuity. As in the preceding experiments, there are minor differences between the results obtained with the glutens from the two flours, but the relation between the percentage of total nitrogen precipitated and the magnesium sulphate concentration is regular, there being no indication of discontinuity.

Fractionation and Analysis of the Fractions

Having shown by the preceding experiments that there is apparently no discontinuity in the solubility behavior of gluten protein, an attempt was made to fractionate the dispersed protein and to determine what chemical differences existed between successive fractions. Dispersions were prepared and made up to 200 ml. Two 5.0 ml. aliquots were analyzed for total nitrogen, and the remainder transferred quantitatively to centrifuge bottles. The dispersions were then adjusted to 0.02 of saturation with magnesium sulphate solution and the precipitated protein separated and washed as described earlier. The supernatant liquid was decanted into a second cen-

trifuge bottle and the washings added. The entire process of adding magnesium sulphate solution, separating and washing the protein precipitate was repeated, and fractions obtained at salt concentrations of approximately 0.025, 0.035, 0.05, 0.07, 0.1, 0.2 and 0.5 of saturation. The nitrogen distribution of these fractions was determined, and the results are plotted in Fig. 3. The value represented by the point on the extreme right of each of the curves was obtained by difference, as explained later. The amide and arginine nitrogen content are expressed as percentages of the total nitrogen in the respective fractions. Each of these values is plotted at the cumulative mid-point of the fraction for which it was obtained, the mid-points being expressed as percentages of the total nitrogen in the original dispersion.

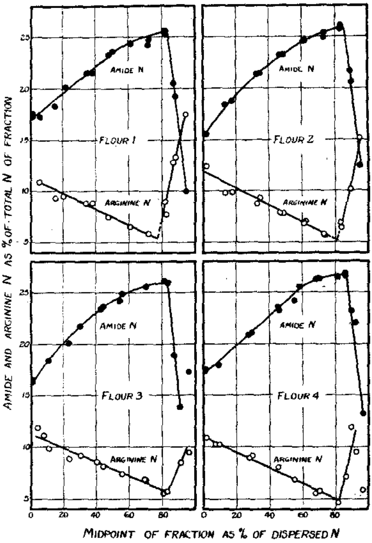

FIG. 3. *Amide and arginine nitrogen content of gluten fractions.*

In this experiment each fraction was exposed to sodium salicylate solution for one day more than the preceding fraction. Consequently, before drawing any conclusions from the results, it was necessary to determine whether exposure to sodium salicylate affected the chemical composition of the protein. Dispersions were prepared using gluten from the four flours and allowed to stand overnight in the refrigerator. After 24 hr. exposure to sodium salicylate two aliquots of each dispersion were pipetted into centrifuge tubes. One of each pair was immediately brought to 0.2 of saturation with respect to magnesium sulphate, and the precipitated protein separated and analyzed as already described. The other aliquot of each pair was stored for a further nine days, a period corresponding to the longest time any of the fractions were exposed, and the protein then precipitated and analyzed as before. The results are given in Table II, each value being an average of duplicates, and show that neither the percentage precipitated nor the chemical composition of the protein was changed significantly by the prolonged exposure

to sodium salicylate. In the "F" columns of Table II are given the corresponding values obtained from the results of the preceding fractionation study, by summing the total, amide and arginine nitrogen of the successive fractions obtained up to 0.2 of saturation with magnesium sulphate. The amide nitrogen results show excellent agreement with those obtained by direct precipitation, as do the arginine nitrogen results for Flours 3 and 4. The arginine nitrogen values for the other flours appear to be low, a discrepancy that can probably be attributed to error.

TABLE II

EFFECT ON GLUTEN OF EXPOSURE TO, AND FRACTIONATION FROM, 10% SODIUM SALICYLATE SOLUTION

Flour	Total nitrogen precipitated, as percentage of total nitrogen in dispersion			Amide nitrogen, as percentage of total nitrogen precipitated			Arginine nitrogen, as percentage of total nitrogen precipitated		
	1	10	*F*	1	10	*F*	1	10	*F*
1	87.5	88.4	85.9	22.0	22.1	22.0	8.7	8.8	8.2
2	87.3	88.6	87.2	22.4	22.0	22.0	8.9	8.8	8.1
3	88.9	89.8	86.8	22.3	22.4	22.6	8.2	8.2	8.2
4	90.7	90.9	88.7	23.1	23.0	23.0	7.7	7.9	7.8

1 *Precipitated after exposure for one day to sodium salicylate solution.*
10 *Precipitated after exposure for 10 days to sodium salicylate solution.*
F *Fractionally precipitated.*

It is impossible to precipitate all the protein in dispersions in sodium salicylate by the addition of magnesium sulphate and unfortunately the usual protein precipitants, which are acidic, cannot be used on account of the insolubility of salicylic acid. The nitrogen distribution of the protein remaining in solution was calculated, however, from the data given in Tables I and II and the average of the corresponding values after 1 and 10 days' exposure of the protein to sodium salicylate was plotted, as previously stated, in Fig. 3. Except for the percentage of arginine nitrogen calculated for the non-precipitated fraction of the gluten washed from Flour 4, these results appear to fit on the respective curves very well. It is probable that the value 7.6% reported in Table I for the arginine nitrogen content of the washed gluten is too low, and a slight error in this value would greatly affect the results calculated for the non-precipitated residue.

In order to check these calculated values by direct analysis the soluble nitrogenous substances removed in washing the gluten from Flours 2 and 4 were hydrolyzed and analyzed as before. It is obvious from the results in Table I that washing would remove about 10% of the total nitrogen in these flours. The amide and arginine nitrogen content, expressed as percentages of the total nitrogen washed from the gluten, were as follows: Flour 2, amide

arginine nitrogen, 17.4%. In each case the amide nitrogen is lower and the arginine nitrogen higher than the value represented by the extreme right-hand point in the respective curves in Fig. 3. This is in direct agreement with the trend of the curves in Fig. 3, and the value 17.4% for the arginine nitrogen content of the nitrogenous material washed from Flour 4 lends support to the contention that the value 5.8% calculated for the non-precipitated fraction is in error.

It is obvious from Fig. 3 that, except for the most soluble 10-15% of the protein in washed gluten, the amide nitrogen content increases and the arginine nitrogen content decreases with increasing solubility of the protein. There are minor differences among the results obtained with the different flours but, apart from small deviations which can be attributed to error, the curves are smooth and regular with no evidence of discontinuity. The most soluble portion of the protein is discussed in a later section.

Examination of Gluten Fractions and Reversibility of Fractionation

Having demonstrated in the preceding experiment that gluten protein could be fractionated into a number of progressively different fractions, an additional experiment was performed in which the fractions were examined and then redispersed, the resulting dispersions mixed, and the protein again salted out and examined. The object was to determine whether the fractions resembled gluten and if not, whether they could be recombined to give a gluten.

In this experiment a dispersion of gluten from Flour 3 was used. Fractions were obtained at 0.025, 0.04, 0.07 and 0.2 of saturation with magnesium sulphate, as previously described, and each fraction was washed with distilled water, those that were not tenacious being repeatedly rinsed and centrifuged, until the wash liquid gave a negative test for sulphate ion.

Fraction 1 contained about 30% of the total nitrogen originally in the dispersion and, on washing, it matted into a tough, tenacious, smooth mass. Fraction 2 came down as a soft gel which contained about 25% of the total nitrogen originally in the dispersion. On washing, the protein exhibited a slight tendency to hold together but could not be matted into a tenacious ball. Fraction 3 contained about 20% of the total nitrogen originally in the dispersion, and precipitated as small particles which showed little tendency to cohere. Fraction 4 contained approximately 15% of the total nitrogen originally in the dispersion and precipitated as a finely divided powder that was not changed during washing.

After examination, the fractions were redispersed in 10% sodium salicylate. Some difficulty was experienced in dispersing Fraction 1, as the tenacious mass would not break up and the dispersing agent appeared to attack the surface only. On transferring the protein mass to a mortar and grinding with sodium salicylate solution, complete dispersion was finally effected. Fraction 2 dispersed with little difficulty and Fractions 3 and 4 seemed quite soluble. The resulting dispersions differed considerably in turbidity which varied progressively from that of Fraction 1, which was quite opaque, to that of Fraction 4, which was almost clear.

The dispersions of the four fractions were combined, mixed thoroughly nd the protein then precipitated by the addition of magnesium sulphate olution to 0.2 of saturation. The protein came down as a tenacious mass hich after washing was indistinguishable from one which had not been reviously fractionated, and distinguishable from freshly washed gluten nly by its freedom from starch and a slightly increased toughness and ecreased elasticity. The precipitated gluten closely resembled the residue f a washed gluten after extraction with water and, as extracting with water lissolves about 10% (Fig. 1) and precipitation at 0.2 of saturation with nagnesium sulphate leaves about 10% (Table II) of the total nitrogen of vashed gluten, this similarity is readily explained.

Discussion

Before discussing the results in general, the break in the amide and arginine curves (Fig. 3) requires explanation. This discontinuity, and the results obtained by analyzing the nitrogenous substances removed in the preparation of washed gluten, indicate that the most soluble protein differs from the remainder. This fraction represents 20-30% of the protein in flour, only about half of which is included in washed gluten as prepared in these experiments. The solubility curves (Figs. 1 and 2) do not exhibit any breaks, which may suggest that this most soluble fraction is not distinct from the rest of the protein on the basis of solubility. The curves in Fig. 2, however, are flattening out in the region where 85-90% of the protein is precipitated. In extracting washed gluten it is impossible, as previously stated, to break up the gluten ball with the lower concentrations of sodium salicylate, so the lower points on the curves in Fig. 1 may not be sufficiently accurate to exhibit a slight break if one did exist.

It cannot be stated from the results of the present study whether this most soluble 20-30% of the protein in flour is one distinct protein, or several, or a protein complex, but this portion appears to be distinctly different from the rest of the protein. The terms "non-gluten" and "salt soluble" are applied to the more soluble protein in wheat flour, and this fraction is commonly, though arbitrarily, as Blish (1) points out, estimated by extraction with 5% potassium sulphate. This salt solution extracted 13.4 and 11.4% of the total nitrogen of Flours 2 and 4, respectively, which is much less than the amount which appears, from the results of the present study, to be distinctly different from the rest.

None of the results of this investigation lend any support to the classical view that gluten is composed of glutenin and gliadin. The results in Fig. 1 show that, by choosing a solution of sodium salicylate of suitable concentration, practically any desired percentage of the gluten can be extracted and that this percentage is approximately constant with the different flours used. The curves in Fig. 1 are regular and if glutenin and gliadin are distinct proteins the curves might reasonably be expected to exhibit a break in the region of 50% extraction. The curves in Fig. 2 are also regular, so on the basis of

solubility in sodium salicylate there is no evidence of the existence of two distinct gluten proteins. This is in agreement with Cook's conclusion (2) based on the precipitation of gluten protein from a dispersion in urea solution.

It is also well known that so-called "glutenin" and "gliadin" differ in chemical composition. The results in Fig. 3 show, however, that by suitably adjusting the magnesium sulphate concentration a number of fractions can be obtained which vary progressively and uniformly in chemical composition. The most soluble 10-15% of the dispersed protein appears to be different and has already been discussed, but there is no evidence of any break in the curves that could be taken to indicate that gluten is composed of glutenin and gliadin or of glutenin, "mesonin" and gliadin as Sandstedt and Blish (14) suggested.

The fact that no break which could be taken to indicate that gluten is composed of so-called "glutenin" and "gliadin" was found in any of the curves in Figs. 1, 2 and 3, might be explained on the basis that one protein is adsorbed on the other to such an extent that extraction with, or precipitation from, sodium salicylate solutions, merely removes fractions containing progressively less of one and more of the other protein. Were such an explanation valid, however, then that fraction which contains the two components in the same ratio as they are present in gluten should, of necessity, have the same physical and chemical properties as gluten. None of the fractions obtained in the preceding experiment exhibited a close similarity to washed gluten. Bearing in mind the fact that Fraction 1 contained the most insoluble 30% and Fraction 2 the next 25% of the total nitrogen in the original dispersion, reference to Fig. 3 and Table I will show that Fraction 2 had approximately the same chemical composition, as determined by amide and arginine analyses, as the original washed gluten. Its physical properties, however, were not at all similar to those of gluten since, as previously stated, it could not be matted into a tenacious mass. The difference in physical properties between Fraction 2 and gluten cannot be attributed to the action of sodium salicylate solution in the former case since, when the fractions were redispersed, combined and the protein precipitated, it came down as a gluten.

A view which will explain the results obtained in this investigation is that gluten, or at least the major portion of it, is a single protein complex which may be separated into a great many fractions which differ progressively and systematically in both physical and chemical properties. The solubility of the fractions and the turbidity of the resulting dispersions indicate that the protein particles decrease in size with increasing solubility. Sorensen (15) regards soluble proteins such as egg albumin and so-called "gliadin" as "reversibly dissociable component systems". The results of the present investigation show that gluten is reversibly fractionable, and in terms of Sorensen's nomenclature, gluten may be regarded as a "highly dissociable component system."

This view is in accord with the results obtained by Gortner, Hoffman and Sinclair (8) in their extraction studies, and will account for some of the wide

deviations in amide and arginine content of so-called "glutenin" preparations, as different methods doubtless yield samples containing different fractions of the gluten complex. For example, the average amide and arginine content of glutenin prepared by Cook and Alsberg (3) from dispersions in urea were respectively 19.5% and 9.8%, while the corresponding values obtained by Larmour and Sallans (10) for glutenin prepared from a dispersion in 0.007 *N* acetic acid were 21.0% and 8.5%. The average amide and arginine content of the most insoluble 40% of the gluten in the present study (Fig. 3) were 19.6% and 9.6%, which values are in good agreement with those obtained by Cook and Alsberg. Larmour and Sallans, however, failed to secure complete dispersion of the gluten in the reagent used, and the undispersed portion was undoubtedly that portion which was lowest in amide and highest in arginine nitrogen. The failure to include this in the prepared glutenin would result in a higher amide and a lower arginine content than would otherwise have been obtained. The differences in the results presented by Cook and Alsberg and Larmour and Sallans are, therefore, explained. Doubtless this view will also explain the variability in the chemical composition of glutenin prepared by other workers.

The results of the extraction studies in this and previous investigations (8) show that definitions of flour proteins based on solubility in a solution such as 70% alcohol are purely arbitrary, as the changes in physical and chemical properties are not sudden but gradual and regular. The precipitation experiment in the present study appears to give a much better picture of the nature and composition of gluten, and it therefore seems preferable that the terms "glutenin" and "gliadin" should be discarded. These suggest that gluten is composed of two distinct proteins, whereas the results of the present study, and those of earlier investigations (2, 8), substantiate the view that it is a single protein complex which can be divided into a great many progressively different fractions.

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DENSITY AND ADSORPTION STUDIES IN THE REGION OF THE CRITICAL TEMPERATURE: SYSTEM DIMETHYL-ETHER–ALUMINA[1]

By J. Edwards[2] and O. Maass[3]

Abstract

An apparatus and an experimental procedure for simultaneously determining the density and adsorption of liquids, vapors and gases in the region of the critical temperature are described. Measurements of adsorption and density of dimethyl ether through the temperature range 124–135° C. are given for the liquid, vapor and gas. Adsorption of the liquid does not occur below 124° C. but beginning at this temperature, it rapidly increases to a maximum, beyond which the adsorption curve resembles a normal isobar. It is shown that the apparent adsorption cannot be ascribed to a lag in the critical temperature of liquid held in the pores, and further that an absurd value for the density of such liquid must exist to account for the total adsorption observed. Hysteresis effects of a complex nature are noticed, and these are discussed from the point of view of the hypothesis that: "there is an essential difference, other than concentration, between a liquid and a highly compressed gas."

Introduction

The series of researches that has been made in this laboratory on the behavior of liquids and gases in their critical regions was recently extended by Morris and Maass (8) to the study of adsorption for the system propylene–alumina. Inasmuch as the results of this investigation were of sufficient interest to warrant a more exhaustive survey of the phenomenon of adsorption at high pressures, and particularly in the region of the critical temperature and pressure, adsorption isotherms from −78 to 130° C. through a pressure range 0.1–52.6 atm. were determined for the system methyl-ether–alumina. The results of this investigation will be published by the authors in a later paper.

On account of the large error involved in the estimation of the density of the methyl ether surrounding the gel above the critical temperature, the buoyancy corrections applied to the apparent weight of the gel yielded values for the adsorption which were obviously greatly in error. An apparatus was therefore constructed which would allow simultaneous determinations of density and adsorption to be made under all the experimental conditions imposed. Quite apart from the significance which this method gave to the adsorption data, the variation of density with temperature under a variety of conditions proved to be of considerable interest in the further elucidation of the nature of the liquid-gas equilibrium at the critical temperature. In this paper it will be shown that results have been obtained which cannot be satisfactorily explained on the basis of continuity of state.

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Experimental

Description of the Apparatus

The bomb (Fig. 1) consisted of specially selected heavy-walled Pyrex tubing, with the arm *D* of 14 mm. inside diameter and wall thickness 4 mm., while *A* and *B* were of 11.5 mm. inside diameter and 3.5 mm. wall thickness. Connection among the three arms was made with capillary tubing of 1.2 mm. bore.

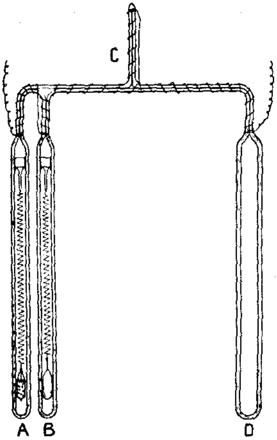

FIG. 1. *The bomb.*

The bomb *A* contained a quartz spiral suspended by a hook from a glass collar which was held snugly in place by a small indentation in the bomb. The alumina gel, contained in an aluminium basket perforated with small holes, was hung from the bottom ring of the quartz spiral by means of a fine aluminium wire fastened to the basket. The gel was prepared according to the method of Munro and Johnson (9) and subsequently heated in a muffle furnace to 600° C. The quartz spiral had a sensitivity of 0.00366 gm. per mm. extension, and the maximum load was 0.50 gm.

The bomb *B* was of the same dimensions as *A*, and differed from *A* only by the substitution of a float for the sorbent. The sensitivity of the spiral was 0.00517 gm. per mm. extension. The float occupied a volume of 0.8971 cc. and weighed 0.3769 gm.: the density of the float was thus 0.4201. If a liquid of density greater than this surrounded the float, it would rise through and break the spiral; measurements of the density and adsorption of liquid methyl ether were therefore confined to the region above 121° C. Since the adsorption values up to the critical temperature had previously been obtained very accurately by applying the density values of Cardoso and Coppola (2), Cardoso and Bruno (1), and Tapp, Steacie, and Maass (13), this lack of flexibility in the apparatus was of no real concern in the present work.

The connecting capillary tubing was covered with a thin layer of asbestos paper, about which was wound a length of resistance wire. The temperature of the capillary tubing could thus be maintained from 10 to 20° C. above that of either arm.

The total volume of the apparatus was approximately 96 cc., distributed so that the volume of *D* was nearly equal to the combined volumes of *A* and *B*.

Connection was made through *C* to a high-vacuum line and to a fractional distillation unit. The complete lengths of *D*, and *A* and *B* up to the top of

the gel and float were maintained at a temperature of 360° C. while the system was alternately evacuated and flushed with air dried over phosphorus pentoxide. The dry, evacuated system was then flushed a number of times with methyl ether, and an amount sufficient to give a meniscus disappearing near the top of *D*, or, alternatively, of *A* and *B*, was condensed into *D*. The methyl ether was a thrice fractionated portion of 99.95% pure stock supplied in a steel cylinder by Ohio Chemicals Ltd. The bomb was sealed off at *C* and the arm *D* immersed in liquid air while *A* and *B* were held at a temperature of 140° C. Readings of the spiral extensions in both of these bombs then gave a check on the weights of the float and gel determined previous to assembly of the apparatus. Though the weights of the float as determined by both methods agreed within less than 0.05%, the weight of the gel as determined by spiral extension was appreciably lower than the original weight. This loss in weight was attributed to desorption of water vapor during the process of alternate evacuation and flushing with dry air described above. The actual weight of sorbent as determined after assembly of the apparatus was 0.2829 gm., which included 0.0473 gm. of metallic aluminium comprising the basket and wire. The volume of the complete unit was 0.0842 cc. This value was obtained from the known density of aluminium and the density of the gel measured by water displacement. The latter value was 3.44 gm. per cc.

The apparatus was immersed in two thermostats so that the temperatures of *D* and of *AB* could be separately varied and controlled. (In future the side of the apparatus containing the gel and float will be designated "*AB*", while the other side will be referred to as "*D*"). The thermostats consisted of Pyrex jars 10 in. in diameter and 18 in. in height, free from striations in the walls, and filled with dibutyl phthalate to a level about one inch below the horizontal capillary tube connecting the arms. This liquid possessed the advantages of transparency and low volatility at the temperatures required. Suitable heaters, copper cooling coils and stirrers permitted temperature control to within 0.1° C., the limit of accuracy of the calibrated thermometers which were read through a telescope situated alongside the cathetometer. Properly adjusted lighting permitted a clear vision of the ends of the spiral.

The complete apparatus was encased in a wooden box reinforced with strap iron and containing three built-in shatter-proof glass windows. The possibility of damage to the cathetometer and telescope was minimized by placing a plate glass window directly in front of these instruments.

Experimental Procedure

In general, two procedures were adopted. The arm *D* was held at some arbitrarily fixed temperature above the critical temperature (126.9° C.), *e.g.*, 130.0° C., and *AB* heated slowly from 122 to 135° C. and cooled to 122° C. Readings were taken at approximately 1° intervals. That temperature equilibrium between the thermostat and the contents of the bomb had been attained was assured by following the change in the position of the

float after the thermostat had reached the required temperature. When, after a 10 min. interval, no change in the position of the float was detectable, the spiral extensions in both bombs were determined. In so far as the attainment of true equilibrium in adsorption was concerned, test experiments showed that no change in the position of the basket was detectable after 30 min. at true temperature equilibrium.

The second procedure adopted was the reverse of the one described above. The arm AB was held at a fixed temperature, *e.g.*, 130° C., and the temperature of arm D varied through the range 122–135° C.

Values for the apparent adsorption were obtained by means of the equation

$$W_o = (A-N)S + d_m v_g - w$$

where w = the true weight of the gel, d_m = density of the medium surrounding the gel, v_g = volume of the gel, A = length of the spiral under experimental conditions, N = normal length of the spiral under no load, and S = sensitivity of the spiral. It is seen that the term $d_m v_g$ is the buoyancy correction for the medium displaced by the gel, and that $(A-N)S$ is the apparent weight of the gel.

The expression for W_o, the apparent adsorption, does not take into account the correction due to the buoyancy effect of the adsorbed material itself, so that the true adsorption, $W = W_o + d_m v_a$, where v_a is the volume of the adsorbed phase. Since $v = W/d_a$, where d_a is the density of the adsorbed phase,

$$W = \frac{W_o d_a}{d_a - d_m}.$$

A full discussion of the use of this equation in estimating the true adsorption will be presented later. The total error in the determination of W_o did not exceed 0.0008 gm.

The density of the medium surrounding the float was estimated to within ±0.0005 gm. per cc.

TABLE I

RESULTS OF DENSITY DETERMINATIONS

Temp. of AB, °C.	Density	Temp. of AB, °C.	Density	Temp. of AB, °C.	Density
Arm D at 128° C.					
124.0	0.3740	130.4	0.1834	129.6	0.1904
125.4	0.3599	132.9	0.1687	128.3	0.2155
126.8	0.3132	133.5	0.1633	127.7	0.2623
127.2	0.3070	134.7	0.1597	126.3	0.3365
127.6	0.2850	131.6	0.1753	125.1	0.3557
128.3	0.2280	130.6	0.1866	124.4	0.3641
Arm D at 130° C.					
123.7	0.3805	130.6	0.2287	130.2	0.2355
123.9	0.3762	130.7	0.2280	129.6	0.2630
125.2	0.3559	130.9	0.2233	129.5	0.2701
125.4	0.3573	132.2	0.2015	129.1	0.2816
125.8	0.3499	134.3	0.1824	128.4	0.2989
126.8	0.3345	134.6	0.1784	126.9	0.3346
127.4	0.3274	134.7	0.1766	125.3	0.3584
128.2	0.3074	132.2	0.1973	124.0	0.3755
129.5	0.2744	130.7	0.2194		
Arm D at 127° C.					
125.0	0.3560	127.0	0.2465	129.0	0.1842
126.4	0.3300	128.0	0.1960		

Results

The results obtained for the densities are given in Tables I and II, and in Figs. 2 and 3; and those for the adsorption, in Tables III and IV and Figs. 4 and 5.

Adsorption is expressed in the usual manner as x/m, where x is the weight adsorbed, and m is the weight of adsorbent.

In all the curves, the solid lines represent values obtained in the first half of the experiment, *i.e.*, when temperature was approached from below, while the dotted lines indicate the values obtained during the cooling, or second part of the experiment. The hysteresis obtained in the density curves cannot be explained on the basis of a temperature lag, although such a lag would tend to bring the curves together. In order to demonstrate this, a procedure such as the following was adopted: If in the early part of the experiment, the arm AB was at, say, 127.0° C., and a measurement was to be taken at 128.0°, the arm would be heated to 128.2° and held at this temperature until the float was stationary for a 10 min. interval, and then allowed to cool to 128.0° C. When the float was again stationary, temperature, density and adsorption measurements would be made. In the second half of the experiment the arm would be cooled from a higher temperature, *e.g.*, 129.0° C., to 127.8°, and subsequently reheated to 128.0°. In this manner any temperature lag was in a direction tending to bring the two curves

TABLE II

RESULTS OF DENSITY DETERMINATIONS

Temp. of D, °C.	Density	Temp. of D, °C.	Density	Temp. of D, °C.	Density
Gel and float (Arm AB) at 128° C.					
123.9	0.1493	129.7	0.3150	128.8	0.2933
125.5	0.1646	131.2	0.3277	127.7	0.2239
126.7	0.1870	134.8	0.3460	125.6	0.1665
127.6	0.2172	132.7	0.3378		
128.7	0.2875	130.8	0.3249		
Gel and float (Arm AB) at 130° C.					
123.5	0.1382	130.4	0.2701	129.5	0.2339
125.7	0.1548	131.3	0.2852	127.6	0.1788
127.3	0.1762	134.7	0.3272	125.6	0.1558
128.2	0.1945	132.0	0.2966		
129.5	0.2288	130.5	0.2756		

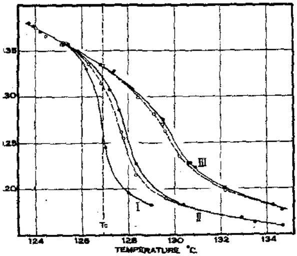

FIG. 2. *Variation with temperature of the density of material in AB. Solid curves: temperature approached from 124° C. Broken curves: temperature approached from 134.7° C. I. Arm D held at 127.0° C. II. Arm D held at 128.0° C. III. Arm D held at 130.0° C.*

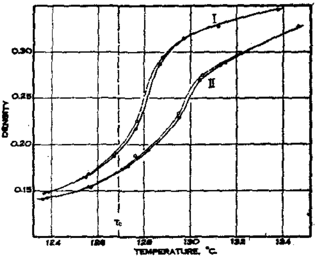

FIG. 3. *Variation, with temperature of arm D, of the density of the material in arm AB. Solid curve: temperature approached from below. Broken curve: temperature approached from above. I. Arm AB held at 128.0° C. II. Arm AB held at 130.0° C.*

together, so that the recorded values indicate the minimum possible hysteresis. Since the error in temperature measurement was 0.1° C., the heating and cooling curves could be made to coincide in the regions of small hysteresis by choosing values within the limits of error. However, the tabulated results were chosen from a large number of observations for which in no case did the hysteresis disappear. The experiments were all repeated from three to five times.

In Fig. 2 the density–temperature relations of arm *AB* are given for three fixed temperatures of *D*. The incomplete and unchecked Curve I has been included to indicate the approximate density behavior in *AB* when *D* is held at the critical temperature. An important consideration arising out of an inspection of Fig. 2 concerns the hysteresis effects in Curves II and III. Tapp, Steacie, and Maass (13), Winkler and Maass (16), and Morris (7) found that the densities existing above and below the level of disappearance of the meniscus differed by a considerable amount at the critical temperature; the density in the region previously occupied by liquid being greater than that in the region previously occupied by vapor. It was further found that on increasing the temperature of the bomb this difference in density rapidly decreased, finally disappearing about 14° C. above the temperature at which the meniscus disappeared, and that on re-cooling, even to the critical temperature, the density difference did not reappear, that is, on re-cooling, the density of material below the level of meniscus disappearance remained steady down to the critical temperature. In the apparatus used in the present investigation the process is complicated by the temperature variations between the two arms, so that a large variation in the distribution of material results. Nevertheless, the hysteresis shown in Fig. 2, Curves II and III, represents an exact parallel to the results of other investigators, and serves to corroborate their data.

It will be seen that the procedure adopted to yield the data in Table II and illustrated in Fig. 3 gives a picture of the behavior of the density of the material in *D* corresponding to Fig. 1 for *AB*. Thus the hysteresis in density in Fig. 2, showing a lower density upon re-cooling, is reflected by a hysteresis in *D* such that a higher density is obtained on re-cooling. This hysteresis is not considered important except in that it corresponds to the different distribution of material between the two arms, and that it gives further evidence for the existence of the hysteresis in Fig. 2.

In Curve II, Fig. 2, it will be seen that the density (solid line) at 128.0° C. is 0.2505, while the corresponding density in *D*, also at 128.0° C., is 0.2420. Under these conditions of equal temperature, the apparatus may be considered as a straight bomb, *AB* representing the region below the point of disappearance of the meniscus. Thus the density difference obtained by other investigators is again confirmed. That this density difference decreases rapidly with increasing temperature may be seen by comparing Curve III, Fig. 2 and Curve II, Fig. 3. For instance, the density of material in *AB* at 130° is 0.251, while the density of material in *D*, also at 130° C., is 0.2505.

Although the decrease in density difference is somewhat greater than would be expected by comparison with the results obtained by Tapp, the parallelism between the two sets of results is a convincing substantiation of their reliability.

The change in density of liquid below the critical temperature appears, from Fig. 2, to be dependent on the temperature of bomb *D*. The possible significance of this fact, together with the nature of the curves above the critical temperature will be fully discussed later in the paper.

The results of the adsorption measurements are given in Tables III and IV and in Figs. 4 and 5. Adsorption is W_o per gram of adsorbent. In Fig. 4, the apparent adsorption, plotted against temperature of the arm *AB*, corresponds to the density curves of Fig. 2, while Fig. 5 corresponds to Fig. 3. It will be noticed (Fig. 4) that the adsorption of liquid methyl ether up to 124° C. is zero, and that adsorption, which begins at about this temperature, increases with increasing temperature, reaching a maximum at a temperature approximately 0.3° C. higher than the temperature of arm *D*. From this point the adsorption-temperature curve resembles the normal gas or vapor adsorption isobar. The second, or cooling part of the experiment, yields a complex

TABLE III

Results of adsorption determinations

Temp. of *AB*, °C.	Adsorption	Temp. of *AB*, °C.	Adsorption	Temp. of *AB*, °C.	Adsorption
Arm *D* at 128° C.					
124.0	0.0006	130.4	0.0251	128.3	0.0267
125.4	0.0046	132.9	0.0201	127.7	0.0187
126.8	0.0095	134.7	0.0173	126.3	0.0053
127.6	0.0134	133.5	0.0210	125.1	0.0028
128.3	0.0283	130.6	0.0255		
Arm *D* at 130° C.					
124.2	0.0018	130.9	0.0268	130.2	0.0226
125.6	0.0057	132.0	0.0222	129.1	0.0191
128.3	0.0125	132.7	0.0216	128.4	0.0130
128.8	0.0138	134.7	0.0187	126.4	0.0065
129.5	0.0188	132.5	0.0237	124.3	0.0007

Arm *D* at 127° C.

Temp. of *AB*, °C.	Adsorption	Temp. of *AB*, °C.	Adsorption
124.8	0.0000	128.0	0.0283
125.9	0.0104	129.0	0.0255
127.0	0.0180		

TABLE IV

Results of adsorption determinations

Temp. of *D*, °C.	Adsorption	Temp. of *D*, °C.	Adsorption	Temp. of *D*, °C.	Adsorption
Gel and float (Arm *AB*) at 128° C.					
123.6	0.0241	132.0	0.0108	127.2	0.0295
125.7	0.0279	134.7	0.0058	126.4	0.0317
127.0	0.0288	131.3	0.0127	125.6	0.0270
128.3	0.0198	129.5	0.0167		
129.6	0.0149	128.3	0.0242		
Gel and float (Arm *AB*) at 130° C.					
123.5	0.0162	129.5	0.0215	129.5	0.0226
125.7	0.0208	130.4	0.0180	127.7	0.0230
127.3	0.0224	131.3	0.0166	126.4	0.0223
128.2	0.0225	134.7	0.0085	125.1	0.0197
128.3	0.0227	130.5	0.0194		

type of hysteresis which cannot be satisfactorily explained on the basis of a lag in equilibrium. Thus from 134.7° C. to 131 or 132° the adsorption is greater than that obtained earlier, while the curve has a negative slope. Provided equilibrium had not been attained, the adsorption values at true equilibrium would result in a greater hysteresis than has been indicated. The second portion of the cooling curve, on the other hand, can be explained on such a supposition, but it appears unusual that such behavior should be apparent in a narrow region only. The hysteresis at low temperature can also, of course, be explained by a lag in equilibrium. An interpretation of these curves predicting their form and the hysteresis as found will be presented later.

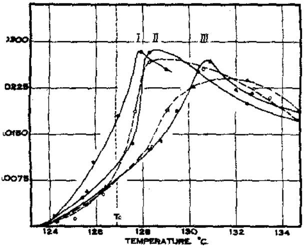

FIG. 4. *Variation with temperature of the adsorption from material in AB. Solid and broken curves have the same significance as in Figs. 1 and 2. I. Arm D held at 127.0° C. II. Arm D held at 128.0° C. III. Arm D held at 130.0° C.*

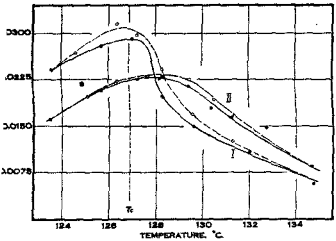

FIG. 5. *Variation of the adsorption from material in AB with temperature of arm D. Solid and broken curves have the same significance as in the previous figures. I. AB held at 128.0° C. II. AB held at 130.0°C.*

In Fig. 5 the situation is much clearer. To begin with, the curves are adsorption isotherms, the gel being held at 128 and 130° C. The low-temperature (of *D*) part of the curve merely represents the normal adsorption–pressure curve, the pressures being the vapor pressures of liquid methyl ether from 124° C. to the critical temperature. The change in slope of the curve above the critical temperature will be treated later, as also will the hysteresis, which is not nearly as complex as in Fig. 4.

Discussion

Density

The primary object of this investigation was a study of adsorption at the critical temperature, but inasmuch as the density measurements recorded concurrently are of interest in themselves, some consideration may be given them at this stage. In the first place they confirm previous work carried out in this laboratory on the persistence of density differences above the critical temperature. In the second place density differences in the liquid below the critical temperature, depending on experimental conditions other than temperature, have been recorded for the first time.

For clarity of discussion, a hypothesis of the nature of the liquid state to explain these and related phenomena, and which will be elaborated more fully later on, may be stated here:— "There is an essential difference other than concentration between a liquid and a highly compressed gas. This difference may be ascribed to a structure in the liquid, whereas in a gas a completely chaotic distribution of molecules exists."

From the hysteresis obtained in the density values it is seen that the density of the material in the region previously occupied by the liquid depends on the extent to which the liquid is heated above the critical temperature (Fig. 2). Both in nature and magnitude of the effect this observation agrees with the results obtained in this laboratory by Tapp, Steacie and Maass (13), Winkler and Maass (16), and Morris (7). On the basis of the above hypothesis the density phenomena up to 14°C. above the critical temperature may be ascribed to the persistence, in part at least, of the structure of the liquid. This persistence was shown to be unimpaired by stirring of the material. On cooling from a temperature appreciably above the critical the re-appearance of the structure in the above sense does not occur until the critical temperature is passed; hence the hysteresis (Fig. 2, Curves II and III).

Fig. 2 shows that density differences in the liquid are appreciable for a full degree below the critical temperature. The condition bringing about a decrease in density is the lower temperature of the arm *D*, the temperature of which governs the amount of material held in it. This immediately raises the question whether the additional weight of material above the point in the liquid where the density is recorded might bring about such a result. It was observed that the maximum difference in height of liquid was only 5%. It is true that, using the calculations by Gouy (4), a 10% difference in density would follow from an increase of 5% in height of liquid, provided the measurements were made within 0.001° C. of the critical temperature, but, as Gouy observed, at 0.1° C. below the critical temperature, the gravitational effect should produce no appreciable density difference, whereas, as will be seen from Fig. 2, a 10% difference in density exists at 0.5° C. below the critical temperature. As this has been recorded for the first time and the apparatus was not suitably designed for very accurate measurements in this region, further discussion of this particular result will await more complete experimental evidence. The meniscus level was always above the top of the spiral, so that while a second order correction to the density values due to the buoyancy effect on the spiral should be made, the relative density values would be unaffected.

The shift in the curves with increasing temperature of the arm *D*, and especially the manner in which the inflection at the equalization of temperature decreases with increasing temperature of *D* are interesting. Since the temperature of *D* governs the distribution of material between the two arms, it was to be expected that the curves should be shifted in the manner illustrated, although it would be logical to expect no inflection in the curve. The meaning of this inflection may be closely linked with the sharp maxima obtained in the adsorption measurements from which a clearer picture of the true state of affairs can be deduced. These will be discussed later.

Regarding Fig. 3, the experimental conditions involved no change from liquid to gas, but rather from vapor to gas, so that no particular interest attaches itself to the form of these curves. They were necessary, of course, for the calculation of the adsorption, and otherwise merely show the distribution of material between the two arms.

Adsorption

The most interesting observations made on adsorption involved the transition of liquid to gas, the results of which have been given in Table III and Fig. 4. A number of arguments may be put forward to account for the nature of these curves, and these will be the first object of discussion. In the first place the curves represent a distinct discontinuity in state in the region of the critical temperature. From the point of view of Le Chatelier's principle, adsorption will always decrease with temperature since adsorption is accompanied by the evolution of heat. Actually a region is seen to exist in which a positive adsorption-temperature derivative may be obtained. Provided, then, that these curves are a true measure of the adsorption, definite statements may be made regarding the magnitude of the homopolar attractive forces between the molecules of sorbate in the vicinity of the critical temperature. In the liquid below 124° C. these forces exceed the attractive forces between sorbate molecules and surface of sorbent. For higher temperatures this is no longer true, so that adsorption becomes apparent. The obvious explanation lies in a change in the nature of the molecular attractive forces at the critical temperature.

Following the region through which adsorption increases with increasing temperature there is seen to be a region in which adsorption falls off with temperature identically as does a normal adsorption isobar. The cooling part of the experiments yields complex hysteresis effects which, together with several other points of importance, will be dealt with later.

The question may now be raised whether the curves in Fig. 4 represent the true adsorption. It must be remembered that the apparent adsorption, W_o, does not take into account the buoyant effect of the adsorbed phase itself, and that the true adsorption, W, is given by

$$W = \frac{W_o d_a}{d_a - d_m} \cdot$$

The density of the adsorbed phase will depend to some extent on the density of the medium and furthermore will decrease slightly with increasing temperature, but the total change in d_a should be very small over a narrow temperature range. Hence the validity of the curves in Fig. 4 may be tested by estimating W and calculating d_a from the above equation.

Let us assume that no discontinuity in true adsorption accompanies the transition of liquid to gas. To get an approximate value of W in the region of 124° C. the normal isobar part of the adsorption curve may be extrapolated to 124° C., and from the resulting values of W and the known values of W_o

and d_m, the change in d_a with temperature may be shown as in Fig. 6. It will be noted that a distinct discontinuity at the critical temperature is obtained. Thus, assuming that no discontinuity in adsorption exists, there results a discontinuity in the density of the adsorbed phase, a condition which cannot be logically expected under any circumstance.

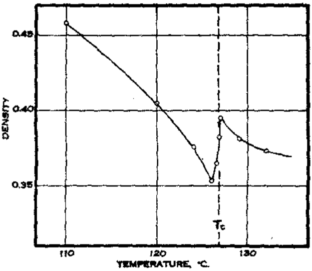

FIG. 6. *The necessary variation of the density of the adsorbed phase with temperature on the assumption that adsorption from liquid and from gas is continuous. Results calculated from Curve II, Fig. 4.*

A more direct method of attack lies in the determination of the true volume of the adsorbed phase, and although this cannot be estimated directly, the pore volume of the gel is fairly accurately known. McGavack and Patrick (6) hold with the hypothesis that all of the sorbed material in a porous body is liquid condensed in the pores. Earlier work by the authors on the adsorption of methyl ether below the critical temperature showed that, in accordance with Patrick's results, the adsorption tended to be independent of temperature at equal relative pressures, p/p_s, where p_s is the vapor pressure of the liquid.

While the Kelvin equation may be used to obtain the radius of the pores, the pore volume may be estimated only by extrapolation of the adsorption to the saturation pressure. Inasmuch as the extrapolation was a small one, and the adsorption was fairly independent of temperature at equal relative pressures, $W_{saturation}$ could thus be estimated to well within 10%*.

Assuming that the liquid condensed in the pores has a density equal to that of bulk liquid at that temperature, the volume of the pores may be obtained as $v_p = W_s/d_l$. The value obtained was 0.09 cc. per gram of gel.

If now we consider the form of the adsorption curves in Fig. 4 to be due to a lag in the critical temperature of material in the pores, the density of this material can be calculated from the known values of W_o, d_m, and v_a (v_a = volume of the pores). The first calculation is the true adsorption, and it is immediately seen that in this case W is obtained by means of Definition A of Coolidge (3) in which the volume of the gel is $\phi_1+\phi_2$, *i.e.*, the volume of "skeleton" plus the pore volume. The resulting curve, corresponding to Curve II, Fig. 4, is given in Fig. 7. Assuming the material adsorbed to be all confined in the pores as liquid, the density of the material is given by $W/0.09$, so that a discontinuity in density of the liquid in the pores would have to be admitted. Considering that W_o was consistently zero below 124° C., the true adsorption according to Definition A of Coolidge must be considered as paralleling the liquid density curve in the region below 124° C.

* *The extrapolations were made for isotherms at 110, 98, 82, and 70° C., the final value of the pore volume being consistent to less than 5%.*

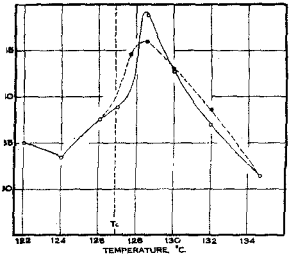

FIG. 7. *Variation of adsorption with temperature, taking the total volume of the gel as volume of the skeleton structure plus pore volume. Calculated from Fig. 4, Curve II.*

Obviously liquid contained in the pores when the gel is surrounded by material of the same density cannot be considered as sorbed—the medium merely wets the gel. Even so, the form of the curve above the critical temperature cannot be explained by persistence of liquid in the pores above the critical temperature. That this statement is true can best be demonstrated by observing the necessary discontinuities in the density of the adsorbed phase at 124° C. and 128° C. (Fig. 7).

It may be definitely shown in another way that the observed adsorption above the critical temperature cannot be due to the persistence of liquid in the pores. In the region below 124° C. the gel is merely wet by the liquid, so that the density of material in the pores is equal to the density of the bulk liquid. Assuming that above a certain temperature near the critical one, the change in density of material in the pores lags behind the rapidly decreasing liquid density, it must be admitted that the density of material in the pores still decreases with increasing temperature. By extrapolation of Fig. 4, Curve II, to 124° C. in the same manner as for Fig. 6, a value may be obtained at 128 or 130° C. which will lie near the density of the material held in the pores. In this way an approximate relation for the variation of the density of adsorbed material with temperature may be obtained for the range 124–135° C. Applying these values, the true adsorption may be calculated, in which case Fig. 8 is obtained. From the values of W and d_a it is seen that in the region near 124° C. the adsorbed material must occupy a volume 10 times that of the pores, or conversely, occupying the pore volume, the material would have to have a density 10 times the density of liquid at a lower temperature. This is an absurd value again, and we are led to the conclusion that the adsorption curves in Fig. 4 cannot be accounted for, except in small part, by persistence of liquid in the capillaries by virtue of an increased

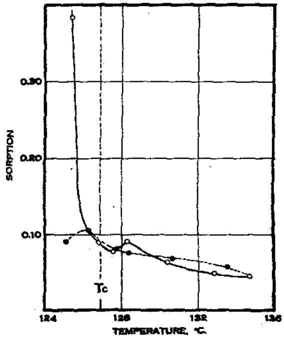

FIG. 8. *Variation of adsorption with temperature, assuming a continuous change in the density of the adsorbed phase. Calculated from Curve II, Fig. 4.*

critical temperature. Further experimental work has yielded interesting points concerning this problem, and in a later paper a full description will be given in the light of the conclusions of Patrick, Preston and Owens (10) that the critical temperature in fine capillary tubes is raised.

It would appear then that, while W_s is not a true measure of the adsorption, an approximate idea of the changes taking place can be obtained from it. Considering the curves in Fig. 4 to be of the form of the true adsorption curve, the hysteresis can be completely accounted for on the basis of the hypothesis stated earlier. There is a persistence of the liquid structure above the critical temperature, and we may conveniently call this the "orientation effect". An accurate measure of how this decreases with temperature has been obtained by Tapp, and Fig. 9 has been reproduced to show how the density difference above and below the region of meniscus disappearance decreases with temperature. This density difference may be used as a measure of the orientation remaining at one temperature. Hence when the material in the region previously occupied by liquid is heated to 135° C. the orientation has largely disappeared, and since, on cooling, it does not reappear until the critical temperature has been passed, the material may be regarded as gas-like in properties—*i.e.*, the molecular motion is more chaotic on cooling than on heating. Since adsorption from the true liquid is zero, and that from the vapor or gas is large, we must expect a hysteresis curve showing greater adsorption.

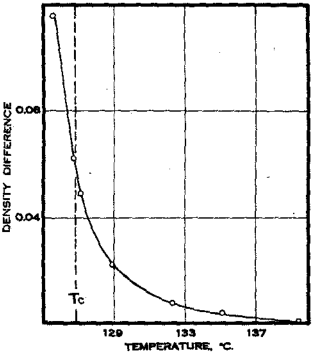

FIG. 9. *Relation between the difference in density above and below the position of meniscus disappearance and the temperature. Reproduced from Tapp, Steacie, and Maass (13).*

A second consideration is the density of the medium. The adsorption will be proportional to the concentration of sorbate molecules, and in the intermediate temperature range of any of the curves in Fig. 4, this concentration is lower during cooling than upon heating, so that a decreased adsorption must be expected. The fact that the hysteresis curve lies above the solid curve shows the influence of the orientation effect, while the intermediate region of lower adsorption on cooling shows that the density effect is greater than the orientation effect.

In the experiments from which the results shown in Fig. 5 were obtained, no liquid-to-gas change was brought about, and the hysteresis curve lies above the plain curve throughout the complete temperature range. This is due to the fact that the concentration of sorbate during the cooling part of the experiment was greater than it was before.

A final comment may be made on the form of the curves (Figs. 2 and 4) in the region of temperature equalization between the two arms. From the inflection in the density curve and the abrupt increase in adsorption it appears that the decrease in liquid structure becomes very great in this region. Why this should be is difficult to explain, but it is highly probable that the expansion of material from one arm to the other, when the temperature of the first passes through that of the second, greatly influences the destruction of residual orientation existing in the material.

Summary

1. The difference in density above and below the region of disappearance of the meniscus observed by other investigators has been confirmed both in nature and magnitude by an entirely different experimental procedure.

2. Adsorption studies in the region of the critical temperature have indicated that a discontinuity in adsorption accompanies the transition of liquid to gas.

3. It has been demonstrated that the apparent changes in adsorption cannot be ascribed to changes in the density of the adsorbed phase or to persistence of liquid in the pores of the sorbent above the critical temperature, and furthermore, there is no evidence of a lag in critical temperature existing for such material.

4. The following hypothesis to account for the phenomena observed is proposed:— "In the liquid state a certain amount of molecular orientation exists. The nature of this orientation may be pictured by considering a sphere of radius equal to the average distance between the centres of molecules in any part of the liquid. The molecules in such a sphere have on the average a definite orientation relative to one another. If a radius twice as great is chosen the molecules may still have a resultant mean orientation but of smaller magnitude; thus with increased radius the average orientation rapidly diminishes. A liquid has therefore a tendency to form a structure into and out of which the molecules move. Increased temperature decreases the number momentarily in the structure, and at the critical temperature this structure no longer corresponds to the minimum potential energy of the system due to heat energy (translational, rotational, and vibrational) of the molecules. This structure has a tendency to persist above the critical temperature,and in a sense may be regarded as metastable, though efficient mechanical agitation has no appreciable effect. A discontinuity in properties other than that due to concentration exists at the critical temperature. Thus the total surface energy is not zero (15), the velocity of a chemical reaction may undergo a discontinuous change (12), and the adsorption from liquid and that from gas may be very different".

The conception of the liquid state given above is not a new one (5, 14), though it has been held that the structure should become zero at the critical temperature. Contributions to this subject have been made by Stewart

(11) through a study of the X-ray diffraction caused by liquids, and the extension of this field to include critical conditions should lead to interesting results.

Preliminary observations on meniscus behavior have indicated that the temperature and position of reappearance of a meniscus is dependent on the previous history of the material contained in the region previously occupied by liquid. Further experimentation along these lines has yet to be completed, but it appears that a meniscus reappears at a higher po ition, and at a temperature less below the critical, when the contents of the bomb are not previously heated appreciably above the critical temperature, so that the ease of formation of liquid is proportional to the residual orientation.

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THE THERMAL CONDUCTIVITY OF DEUTERIUM[1]

By A. B. Van Cleave[2] and O. Maass[3]

Abstract

The thermal conductivities of deuterium and some mixtures of deuterium and hydrogen have been measured by a relative, "hot wire" method. The results are consistent with the authors' original conclusion that the deuterium molecule has the same molecular diameter as the hydrogen molecule. It follows also that the molecular heats of the hydrogen isotopes are the same.

Introduction

In a recent publication (7) the authors gave results for the coefficient of viscosity of deuterium and deuterium-hydrogen mixtures which indicated that the hydrogen isotopes have the same molecular diameter. The work described here was carried out to obtain additional support for the foregoing observation.

Farkas and Farkas (2) have performed experiments on the thermal conductivity of deuterium-hydrogen mixtures, but they were primarily interested in devising a method of analysis for mixtures of the isotopes, and their results cannot be used to make calculations of the thermal conductivity of deuterium.

Experimental

Owing to the large number of somewhat uncertain corrections to be applied, measurements of the absolute thermal conductivity of gases are very inconsistent and difficult to obtain. For this reason a relative method was employed.

The apparatus was modeled after that used by Palmer and Weaver (4). A platinum wire 0.07 mm. in diameter and about 10 cm. in length was stretched along the axis of a cylindrical brass tube of 4 mm. radius. The wire was kept under tension by means of a helical spring of copper wire. One end of the wire was electrically insulated from the metal chamber by attaching it to the terminal of an ordinary spark plug which had been fitted on to the end of the brass cylinder. The cell was provided with an inlet tube which could be connected to the deuterium container, air, hydrogen generator or pumps as desired. It was mounted vertically in an oil bath maintained at 25.00 ± 0.10° C. by means of a thermostat.

The electrical arrangement took the form of a Wheatstone bridge. The thermal conductivity cell and an ammeter formed one arm of the bridge. The corresponding arm, which consisted of a coil of copper wire of about one ohm resistance, was also immersed in the thermostat. Two variable, high resistances made up the remaining arms of the bridge. One of these

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resistance boxes could be varied to within 0.1 ohm. The current was supplied by a bank of dry cell batteries and could be varied by means of resistances of 66 ohms and 1 ohm connected in series.

Assuming that the loss of heat by radiation, by conduction through the connections to the ends of the wire, and by thermal convection is constant, the relative amounts of power expended in maintaining a given temperature difference between the wire and the walls of the chamber, when filled with various gases, affords a measure of the relative thermal conductivities of the gases.

The heat loss, g, from an electrically heated wire is given by

$$g = Ji^2rt,$$

where J is a constant of proportionality, i the current, r the resistance, and t the time of flow. Since in any one experiment the temperature, and therefore the resistance, of the wire is kept constant, the only quantity that need be measured is the current i. The amount of heat given out to the thermostat in unit time is directly proportional to the thermal conductivity of the gas between the wire and the metal tube, and to the square of the current which must be used to keep the temperature of the platinum wire at the same value for all the gases concerned.

If the thermal conductivity of hydrogen be taken as unity, that of any other gas may be found by measuring the currents which will maintain the platinum wire at the same temperature, first in hydrogen and then in the gas of unknown conductivity. That is,

$$k_{gas} = k_H \frac{i^2_{gas}}{i^2_H},$$

where k_H is the thermal conductivity of hydrogen, i_H the current in hydrogen, i_{gas} the current and k_{gas} the thermal conductivity of the gas under consideration.

The cell was first filled with pure dry hydrogen. With the current and one of the variable resistances kept at definite values, the other variable resistance was adjusted until the bridge was balanced. After noting the values of the current and resistance for the balanced condition the first resistance was reset and the procedure repeated. The current was changed slightly and the process again repeated. The apparatus was then filled with deuterium (98% by weight) and, with the variable resistance arms at the same values as found for hydrogen, the current through the wire was varied until the bridge was balanced, thus indicating that the wire had assumed exactly the same resistance as it had in hydrogen. This procedure was repeated for each of the deuterium-hydrogen mixtures. These mixtures were prepared as described in the previous paper (7).

The ammeter which measured the current flowing through the wire could be read to ± 1 milliampere, and was found to give relatively correct values over the range of current used.

For all measurements the gas pressure was one atmosphere.

As a test of the accuracy obtainable the relative conductivities of hydrogen and air were measured and found to agree within 1% with values recently obtained by the above method.

Results

Table I is a summary of the currents necessary to maintain the platinum wire at the temperature corresponding to current readings of 0.2680, 0.2900, 0.3175 and 0.3430 amp. in pure hydrogen. Each of these values is the mean of four independent settings of the variable resistance arms.

TABLE I

VARIATION OF HEATING CURRENT WITH CONCENTRATION

Weight of hydrogen, %					
100	57.05	32.90	14.06	7.74	2.00
Current, amp.					
0.2680	0.2571	0.2465	0.2355	0.2307	0.2280
0.2900	0.2793	0.2670	0.2555	0.2500	0.2460
0.3175	0.3030	0.2905	0.2790	0.2730	0.2680
0.3430	0.3300	0.3150	0.3010	0.2960	0.2890

Table II shows the ratio between the thermal conductivities of various deuterium-hydrogen mixtures and hydrogen as determined from the relation

$$\frac{k_{gas}}{k_H} = \frac{i^2_{gas}}{i^2_H}.$$

TABLE II

VARIATION OF $\frac{i^2_{gas}}{i^2_H}$ WITH CONCENTRATION

Weight of hydrogen, %					
100	57.05	32.90	14.06	7.74	2.00
i^2_{gas}/i^2_H					
1.0000	0.9225	0.8460	0.7722	0.7410	0.7238
1.0000	0.9276	0.8477	0.7762	0.7431	0.7196
1.0000	0.9108	0.8372	0.7722	0.7393	0.7125
1.0000	0.9261	0.8434	0.7701	0.7448	0.7100
Mean 1.0000	0.9216	0.8435	0.7727	0.7420	0.7165

Within the error of this determination the ratio of the thermal conductivities of deuterium-hydrogen mixtures and hydrogen is the same for the four temperatures at which measurements were made. These results indicate that the temperature coefficients of the thermal conductivity of deuterium and hydrogen are the same, as would be expected. The following calculations have been made on this basis.

Discussion

Before discussing the foregoing results it is desirable to refer to some of the conclusions drawn in the previous paper (7) in regard to deuterium-hydrogen mixtures. It was pointed out that complete agreement between experimental results and calculations based on theoretical formulas for the viscosity of gas mixtures was not found, and that a complete discussion of this would be left until a purer sample of deuterium had been obtained. The necessity for this is much more apparent when it is considered that HD molecules are formed during the preparation of D_2 from 98% D_2O, thus decreasing the molecular percentage of D_2. A calculation* shows that the molecular percentages of D_2 and HD in the original sample were 92.54 and 7.06 respectively. This does not alter the conclusions reached with regard to the value of the viscosity of pure deuterium.

If it is assumed that HD molecules have the same molecular diameter as H_2 molecules, the viscosity of pure HD is 1.225 times that of pure H_2. The original sample, which consisted almost entirely of D_2 with HD molecules rather than H_2 molecules as an impurity, was found to have a viscosity of 1.400 referred to the viscosity of hydrogen as unity. A straight line extrapolation for these values of 100% HD and 7.06% HD gives the relative viscosity of 100% D_2 as 1.414 ± 0.003, which is exactly the same as that previously reported (7).

This result may also be obtained by using Pulùj's relation (5) for the viscosity of a gas mixture as an interpolation formula, the value being 1.415 ± 0.003.

It might be argued that since the HD molecule is asymmetrical it would tend to have an effectively larger collisional area than H_2. Assuming this to be the case, it would of necessity follow from the extrapolations that the D_2 molecule has a smaller molecular volume than H_2. This does not seem possible. It follows, therefore, that the previous results (7) indicate that H_2, HD and D_2 molecules have the same molecular diameter to within 0.20%.

The thermal conductivity measurements lead to the same conclusion. From the well known Maxwellian expression

$$k_o = \epsilon \eta_o C_v,$$

where k_o is the thermal conductivity of a gas at 0° C., η_o is the corresponding coefficient of viscosity, C_v is the specific heat at constant volume, and ϵ is a constant, it is possible to estimate k_o for D_2 and HD. Reasons have been given above for believing that D_2, HD and H_2 molecules have the same molecular diameter, hence their viscosities will be in the ratio 1.414 : 1.225 : 1.000. According to Farkas and Farkas (2) the molecular heats of D_2, HD and H_2 are the same at temperatures above 0° C., hence their specific heats will be in the ratio 0.500 : 0.666 : 1.000. For isotopes the constant ϵ should be the same. Hence, the ratio of the thermal conductivities of D_2, HD and H_2 should be 0.707 : 0.817 : 1.000.

* *The authors are indebted to Dr. H. C. Torrey of Columbia University for bringing this calculation to their attention.*

The experimental value for the relative thermal conductivity of the mixture containing 92.54 molecular per cent of D_2 and 7.06 molecular per cent of HD is 0.7165. On the hypothesis mentioned above, the thermal conductivity of HD is 0.817 referred to H_2 as unity. A straight line extrapolation of these two values gives the relative thermal conductivity of pure D_2 as 0.708 ± 0.007, which checks exceedingly well with the theoretical value deduced above. Since the thermal conductivities of D_2 and HD are certainly not very different (2), the error in this extrapolation will be well within the experimental error. Since these results fit so nicely with the results on viscosity the authors believe that this is additional proof that D_2, HD and H_2 molecules have the same molecular diameters and mean free paths at 0° C.

The absolute value of the thermal conductivity of hydrogen at 0° C. is still a somewhat uncertain quantity. The mean of what are, perhaps, four of the most careful determinations (1, 3, 6, 8) with the "hot wire" method gives k_o for hydrogen as 0.000416 cal. cm.$^{-1}$ sec.$^{-1}$ deg.$^{-1}$ C. The maximum deviation from the mean is 0.72%. Relative to the above value of k_o for hydrogen, the authors' value of k_o for deuterium becomes 0.000295 ± 0.000003 cal. cm.$^{-1}$ sec.$^{-1}$ deg.$^{-1}$ C. The calculated value of k_o for HD is 0.000340 cal. cm.$^{-1}$ sec.$^{-1}$ deg. $^{-1}$ C.

Thermal conductivity measurements of other deuterium gas mixtures are contemplated.

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MEASUREMENT OF DIELECTRIC CONSTANT OF ELECTROLYTES[1]

By R. W. McKay[2]

Abstract

An apparatus for measuring the dielectric constant of conducting solutions by voltage resonance is described. The theory of the circuit and the sources of error are discussed. The design of the apparatus is such as to eliminate errors other than those due to inductance of cell leads, for which a correction is made.

Measurements have been made on solutions of sodium chloride, hydrogen chloride, potassium sulphate, magnesium sulphate, copper sulphate and potassium ferrocyanide, at 23.0° C. and 2×10^6 cycles frequency. An increase in dielectric constant with concentration greater than that predicted by Debye and Falkenhagen has been found in all cases. The results are compared with those of other workers. Graphs are given of the results obtained.

1. Introduction

1.1 In recent years striking advances have been made in the theory of electrolytic solutions. Assuming complete dissociation for dilute solutions of strong electrolytes, Debye and others have calculated approximately, not only the thermodynamic functions for equilibrium states, but also such characteristic quantities for irreversible processes, as viscosity, conductivity and dielectric constant. The experimental data available are not sufficient to test the theory in all its phases. This is especially true of dielectric constant measurements. Although nearly 50 years have elapsed since Cohn and Arons made the first measurements of dielectric constant of salt solutions, great discrepancies still exist among the results obtained by many observers, and even the most recent measurements are uncertain. The experimental difficulties which have prevented accurate measurements have been discussed by many authors and complete summaries of the earlier work have been written by Walden and Ulich (19), Lichtenecker (13) and Blüh (1). An extensive bibliography is given by Lattey (12), so that only the most recent work is referred to here.

1.2 The technique of measurement of dielectric constants of insulating materials is relatively simple and several precise methods have been developed for work with such substances. When an attempt is made to extend these methods to conducting materials such as electrolytic solutions, many difficulties arise. Consequently the earlier papers in this field do not contain reliable results, although they give much information which is useful in developing

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more accurate methods. The inconsistency of the results obtained is shown in Figs. 1 and 2, which are plotted from measurements for sodium chloride and copper sulphate by many authors. Some of the more recent papers, however, show much better agreement and appear more reliable. These researches are discussed briefly below.

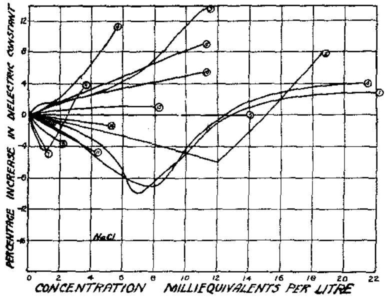

FIG. 1. *Measurements for univalent salts by various authors.* (1) *Millicka and Slama, 50 cycles.* (2) *Schmidt, 500 cycles.* (3) *Smale, 50 cycles (KCl).* (4) *Pechold, 50 cycles.* (5) *Drude, 1 metre; Voigt, 40 cm.; Plötze, 60 cm.; Deubner, 130 cm.* (6) *Malone, Case and Ferguson, 31 metres (1921).* (7) *Lattey.* (8) *Walden, Ulich and Werner.* (9) *Astin, 10^6 cycles.* (10) *Nernst, 10^8 cycles.* (11) *Devoto.* (12) *Lattey and Davies (1931) 4–7.5 $\times 10^7$ cycles.* (13) *Sack, 2 $\times 10^6$ cycles.* (14) *Skancke and Schreiner, 10^6 cycles.* (15) *Jezewski and Kamecki, 5 $\times 10^6$ cycles.*

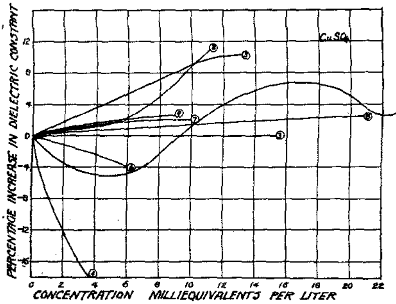

FIG. 2. *Measurements for divalent salts by various authors.* (1) *Schmidt, 500 cycles.* (2) *Smale, 50 cycles.* (3) *Drude, 1 metre; Plötze, 0.40 metre; Voigt, 0.60 metre.* (4) *Malone, Case and Ferguson, 3 metres.* (5) *Lattey and Davies, 10^7 cycles (1932).* (6) *Deubner, 135 cm.* (7) *Wien, 7.5 $\times 10^7$ cycles ($MgSO_4$).* (8) *Wenk 1.1 $\times 10^8$ cycles.* (9) *Jezewski.*

1.3 In the frequency region above 10^7 cycles per sec. the most consistent results have been obtained. Using the original method of Drude, with a Barkhausen oscillator substituted for the Hertzian resonator, Plötze (17) finds that there is no change in dielectric constant for copper sulphate up to 2 millinormal within $\pm 0.3\%$, nor for potassium ferrocyanide within $\pm 0.6\%$ for the same range of concentrations at 0.60 metres wave-length (1.67×10^8 cycles). Drake, Pierce and Dow (4) have modified the method of Drude by substituting a concentric tube and wire for the parallel wire system. In agreement with Plötze they find no change for potassium chloride up to 7 millinormal within $\pm 1.0\%$. The work of Malone, Case and Ferguson (14, 15) is not in agreement with this, but does not appear to be reliable. Stips (18), using the second method of Drude, adapted for very high concentrations, finds that the dielectric constants for normal solutions of copper sulphate and

magnesium sulphate increase *linearly* with concentration up to 10% increase. This is not in disagreement with the results of Plötze or Drake, Pierce and Dow, as the value for small concentrations would be well within the limits of error stated by these authors. Using Wien's "Barretter" method at from 1.5×10^8 to 0.6×10^8 cycles per sec., Wenk (20) and Dobenecker (3) find a slight increase in dielectric constant for the salts they have measured. Wien (21) used the "Barretter" method at 3×10^7, 1.5×10^7 and 1.0×10^7 cycles frequency and found an increase for all salts. The increase becomes larger the longer the wave-length. All these results, except those of Malone, Case, and Ferguson, are substantially in agreement with the theoretical predictions of Debye and Falkenhagen (2).

1.4 For somewhat lower frequencies the measurements do not appear so certain. Lattey and Gatty (12) and Lattey and Davies (10, 11) working at 10 kc., and Jezewski and Kamecki (9) at 5 kc., obtained widely divergent results although the work in both cases was carefully done. The work described in this paper gives results more nearly in agreement with those of Lattey. These are discussed in Section 5.

Graffunder and Weber (6) have also made measurements in this frequency region but the method used is suited only to extremely low concentrations, and even for these it seems doubtful whether any weight can be given to their results. Their method depends on changing the frequency of an oscillator by placing the measuring cell in the oscillating circuit. This method is extremely sensitive but not very accurate.

1.5 Many measurements have been made at very low frequencies using the Force method. Orthmann (16) gives a summary of work by this method up to 1931. In his own work Orthmann used a silver ellipsoid suspended in solutions of silver salts. In this way he believes he has avoided polarization effects. Even in such cases, however, "over-voltage" effects may cause large errors. A. R. Gordon* has shown in some unpublished work that for copper electrodes in copper sulphate solutions, overvoltage exists and is out of phase with the applied voltage at low frequencies. If the same is true for silver in silver salts, errors from this cause would occur in Orthmann's work.

2. Apparatus, Design and Construction

2.1 As the agreement which has been obtained among the results of various workers for the highest frequencies is fairly satisfactory, a somewhat lower frequency (2×10^6 cycles) was chosen for this research. At this frequency the choice of methods is practically limited to the bridge method or the voltage resonance method. Both have been used in this work, but no results of measurements with the bridge are recorded because the resonance method has been found to be much more satisfactory.

**Prof. A. R. Gordon of the Department of Chemistry, University of Toronto.*

2.2 *Bridge Method*

The advantages of a null method are well known. In the case of high-frequency capacity bridges, however, there are also many disadvantages, the most important of which are listed below:—

1. *Insensitivity.* Since almost all alternating current detectors give readings proportional to the square of the input voltage, the sensitivity becomes small as zero reading is reached, making it difficult to balance the bridge accurately. By using an amplifier of sufficient sensitivity it is possible to overcome this difficulty.

2. *Difficulties in shielding.* These are common to all high-frequency measurements, but are somewhat accentuated in bridges because the circuit is more complex than, for instance, in resonance measurements.

3. *Calibration of phase-angle of variable resistances.* Resistances can be made with very small phase angle, but this small remaining phase angle is still sufficient to produce errors in the measurement of capacity of cells containing electrolyte. Various "reactionless" resistances have been devised (see Schreiner, Sack, Weber) but some doubt exists as to the degree to which the reactance has been removed.

These difficulties may be minimized by choosing a suitable bridge circuit. After a careful comparison of numerous bridge circuits, the design shown in Fig. 3 was chosen for preliminary work. It is essentially similar to the Schering bridge used commercially for power factor determinations. It differs chiefly from the standard type in having no variable resistors in the measuring circuit. This change greatly simplifies calibration of the bridge. The use of a Wagner ground arm makes it possible to keep one corner of the bridge at ground potential without connecting it directly to ground. This permits the use of a three-electrode cell (Fig. 4A) so that the field, in the part of the cell which is measured, is entirely in the electrolyte and not through any supporting insulators.

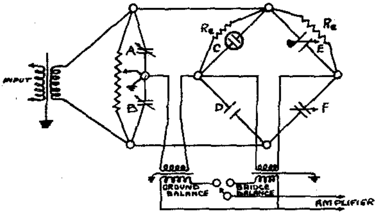

FIG. 3. *Bridge circuit diagram.*

Condensers were used as far as possible for the various units of the bridge arms, since they are more nearly ideal for phase angle than resistances or inductances, and they are also more easily shielded. The theory of the bridge is well known. The value of the capacity in the measuring arm is given by the formula

$$C = \frac{DE}{F},$$

where C is the total capacity of the measuring arm, E that of the comparison arm, and D and F the capacities of the condensers in the other two arms. C is independent of frequency, which is an advantage of this bridge in common with all those of the so-called "perfect balance" type.

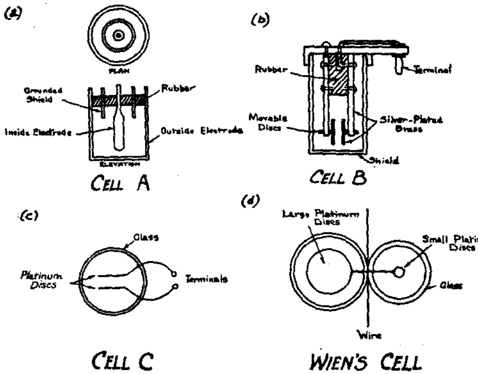

FIG. 4. *Cells.*

The variable condensers E and F each consist of fixed condensers in parallel with a small variable condenser. Their capacity is variable from $5\mu\mu f.$ to 150 $\mu\mu f.$ and can be read at any point to within 0.02 $\mu\mu f.$ They were mounted rigidly in a brass box and shielded from each other. The input from the generator may be varied from zero up to one watt. The detector consists of a three-stage screen-grid amplifier, and a rectifier. The sensitivity is sufficient to balance the bridge within 0.1% when the cell resistance is as low as 100 ohms.

The only point causing trouble in calibration was the evaluation of the shunt capacity of the resistance R_E. The measurement of any resistance of known phase angle suffices to evaluate the capacity of R_E. Various methods tried to find such a resistance are summarized below.

1. Metal films sputtered on glass cylinders might be supposed to have no capacity apart from that of their terminals. This was shown definitely to be untrue for carbon and carborundum in the same geometrical form. A pencil mark on hard rubber was shown to have an effective capacity as high as 10 $\mu\mu f.$ This is probably an anomalous behavior due to the structure of carbon, but one cannot be certain that the same effect is not found to a lesser degree in metal films also. This method cannot, therefore, be relied on.

2. Resistance can be measured accurately on the bridge. If a circuit consisting of resistance, capacity and inductance is adjusted in the bridge till its effective resistance becomes a minimum, it may be shown that for certain circuits the reactance is then zero. Such a circuit provides a method for calibrating R_B. The sensitivity of the method is not great.

3. A shielded resistance was sent to the Bell Telephone Laboratories Inc. (New York) who were kind enough to offer to calibrate it. The calibrated value was 181.13 ohms and $-1.3 \pm .5$ $\mu\mu$f. at 400 kc. Using this resistor the bridge calibration was completed.

The tests which were made with the bridge, the errors in measurement, and the reasons why the bridge was replaced by a resonance circuit are described below in Section 3 (1).

2.3 *Resonance Method*

2.31 *Theory.* The theory of the voltage resonance circuit has been given by Jezewski and by Astin for the case of an isolated resonator, without considering the interaction with the generator and the detector. Lattey considered the interaction with the detector only. Both these interactions may be large enough to affect the results. It is therefore worth while to con-consider the theory of the method in greater detail.

After several other circuits had been tested, the one shown in Fig. 5 was adopted. A quartz crystal oscillator not closely connected to the rest of the

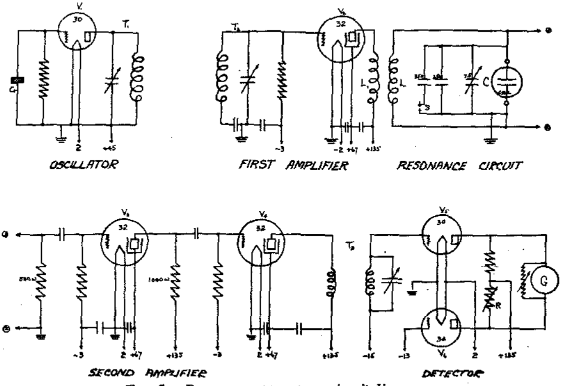

FIG. 5. *Resonance apparatus—circuit diagram.*

circuit supplied a high-frequency current of constant frequency. This current was used to drive a 32-type screen-grid valve, the plate circuit of which was coupled to the coil of the resonance circuit. The resonance circuit was con-

nected to a second 32-type valve which had an impedance of only 1000 ohms in the plate circuit, minimizing the possibility of feed back. These two valves, V_2 and V_3, effectively isolate the resonance circuit from the oscillator or detector, so that for the purpose of calculation the equivalent circuit Fig. 6 may be substituted. The valves V_2 and V_3 are replaced in this diagram by their equivalent networks, in the conventional way. The plate-filament and grid-filament capacities are included in the units l_i, L_1, C and l_o. It is necessary to consider the grid-plate capacities, so they are represented as C_{gp_1} and C_{pg_2} in the diagram. The plate resistances and amplification constants of the tubes are represented by ρ_1, ρ_2 and μ_1, μ_2. It is necessary to assume that the voltage e is harmonic and that the frequency is constant. The experimental justification of these assumptions is given below. In Fig. 6, C_M represents the capacity between the resonance coil L and the plate-circuit coil L_1 between which the mutual inductance is M. The cell capacity is C, and R includes the shunt resistance of the cell as well as the grid resistance of V_3.

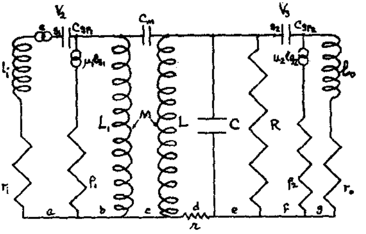

FIG. 6. *Resonance apparatus—equivalent circuit for calculations.*

Kirchoff's equations for the steady state may be written at once in the following form. (The currents i_a . . . . i_g are all taken in the clockwise sense).

$$\left(j\omega l_i + r_i - \frac{j}{\omega C_{gp_1}}\right)i_a - \rho_1 i_b = e - \mu_1 e_{g_1} \quad (1)$$

$$-\rho_1 i_a + (\rho_1 + j\omega L_1)\, i_b - j\omega(L_1 + M)\, i_c + j\omega M i_d = \mu_1 e_{g_1} \quad (2)$$

$$-j\omega(L_1 + M)i_b + j\omega\left(L_1 + L + 2M - \frac{1}{\omega^2 C_M}\right)i_c - j\omega(L + M)i_d = 0 \quad (3)$$

$$-j\omega M i_b - j\omega(L + M)i_c + \left(j\omega L + r - \frac{1}{j\omega C}\right) i_d + \frac{j}{\omega C}\, i_e = 0 \quad (4)$$

$$\frac{j}{\omega C}\, i_d + \left(R - \frac{j}{\omega C}\right) i_e - R i_f = 0 \quad (5)$$

$$-R i_e + \left(R + \rho_2 - \frac{j}{\omega C_{gp_2}}\right)i_f - \rho_2 i_g = -\mu_2 e_{g_2} \quad (6)$$

$$-\rho_2 i_f + (\rho_2 + j\omega l_o + r_o)i_g = \mu_2 e_{g_2} \quad (7)$$

Substitute

$$e_{g_1} = i_a(r_i + j\omega l_i) - e$$

$$e_{g_2} = (i_f - i_e)R$$

Divide Equation (1) by $(1+\mu_1)$ giving Equation (1^1)

Add $\dfrac{\text{Equation (1)}\times\mu_1}{1+\mu_1}$ and (2) giving Equation (2^1)

Add Equation (4) and Equation (5) giving Equation (4^1)

Add Equation (6) and Equation (7) giving Equation (6^1)

Replace the two variables i_d and i_f by new variables

$(i_f - i_e)$ and $(i_e - i_d)$.

This gives

$$\left(j\omega l_i + r_i + \frac{\frac{1}{j\omega C_{op_1}} + \rho_1}{\mu_1+1}\right)i_a - \frac{\rho_1}{\mu_1+1}\,i_b = e \qquad (1^1)$$

$$\frac{\frac{\mu_1}{j\omega C_{op_1}}}{\mu_1+1}i_a + \left(j\omega L_1 + \frac{\rho_1}{\mu_1+1}\right)i_b - j\omega(L_1+M)i_c + j\omega M i_e - j\omega M(i_e - i_d) = 0 \qquad (2^1)$$

$$-j\omega(L_1+M)i_b + j\omega\left(L_1 + L + 2M - \frac{1}{\omega^2 C_M}\right)i_c + j\omega(L+M)i_e + j\omega(L+M)(i_e - i_d) = 0 \qquad (3^1)$$

$$j\omega M i_b - j\omega(L+M)i_c - R(i_f - i_e) + (j\omega L + r)i_e - (j\omega L + r)(i_e - i_d) = 0 \qquad (4^1)$$

$$\left(R + \frac{1}{j\omega C_{op_2}}\right)(i_f - i_e) + \frac{1}{j\omega C_{op_2}}\,i_e + (j\omega l_o + r_o)i_g = 0 \qquad (5^1)$$

$$(-\mu_2 R - \rho_2)(i_f - i_e) - \rho_2 i_e + (\rho_2 + j\omega l_o + r_o)i_g = 0 \qquad (6^1)$$

$$-R(i_f - i_e) + \frac{1}{j\omega C}(i_e - i_d) = 0 \qquad (7^1)$$

Let Δ be the determinant of the coefficients of i_a, etc.

Δ_{ik} be the minor row "i" and column "k" of Δ

D_{ik} be the minor of row "i" and column "k" of Δ_{17}

$$\frac{e}{i_g} = Z_{17} = \frac{\Delta}{\Delta_{17}} = \frac{-\frac{1}{j\omega C}\Delta_{76} + R\,\Delta_{74}}{\frac{1}{j\omega C}D_{66} - RD_{64}} = \frac{-\Delta_{76} + j\omega CR\,\Delta_{74}}{D_{66} - j\omega CRD_{64}}$$

$D_{64} = 0$ identically.

$$\text{So } Z_{17} = j\omega CR\frac{\Delta_{74}}{D_{66}} - \frac{\Delta_{76}}{D_{66}}$$

$$|Z_{17}|^2 = \left(j\omega CR\frac{\Delta_{74}}{D_{66}} - \frac{\Delta_{76}}{D_{66}}\right)\left(-j\omega CR\frac{\Delta_{74}^+}{D_{66}^+} - \frac{\Delta_{76}^+}{D_{66}^+}\right)$$

where Δ^+ represents the conjugate complex of Δ

$$= \omega^2 C^2 R^2\left|\frac{\Delta_{74}}{D_{66}}\right|^2 + j\omega CR\frac{\Delta_{74}^+\,\Delta_{76} - \Delta_{74}\,\Delta_{76}^+}{|D_{66}|^2} + \left|\frac{\Delta_{76}}{D_{66}}\right|^2$$

which is quadratic in C.

For any value C_1 of C another value C_2 will give the same $|Z_{17}|^2$ and therefore the same output current. Since the expression is quadratic in C the mean of C_1 and C_2 will be the same for any corresponding pair of values.

$$\bar{C} = \frac{C_1 + C_2}{2} = \text{resonance capacity} = \frac{j\omega R(\Delta_{76}\,\Delta_{74}^+ - \Delta_{76}^+\,\Delta_{74})}{2\omega^2 R^2\,|\,\Delta_{74}|^2}$$

$$= \text{the imaginary part of } \frac{-\Delta_{76}}{\omega R\,\Delta_{74}}$$

Rearranging the columns slightly and multiplying the fifth row of each determinant by $(\rho_2 j\omega C_{op_2})$ and adding the sixth row to it, the two determinants can be written:—

$$-\frac{\Delta_{78}}{R} =$$

$$\left| \begin{matrix} j\omega l_i + r_i + \dfrac{\rho_1 + \dfrac{1}{j\omega C_{op_1}}}{\mu_1 + 1} & \dfrac{-\rho_1}{\mu_1 + 1} & 0 \\ \dfrac{\dfrac{\mu_1}{j\omega C_{op_1}}}{\mu_1 + 1} & j\omega L_1 + \dfrac{\rho_1}{\mu_1 + 1} & -j\omega(L_1 + M) \\ 0 & -j\omega(L_1 + M) & j\omega\left(L_1 + L + 2M - \dfrac{1}{\omega^2 C_M}\right) \\ 0 & +j\omega M & -j\omega(L + M) \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{matrix} \right.$$

Right half of above———

$$\left. \begin{matrix} 0 & 0 & 0 \\ j\omega M & 0 & 0 \\ -j\omega(L + M) & 0 & 0 \\ j\omega L + r & 0 & 1 \\ 0 & \rho_2 + (j\omega l_o + r_o)(1 + j\omega C_{op_2} \rho_2) & \mu_2 - j\omega C_{op} \rho_2 \\ -\rho_2 & \rho_2 + j\omega l_o + r_o & \mu_2 + \dfrac{\rho_2}{R} \end{matrix} \right|$$

and

$$\Delta_{74} =$$

$$\left| \begin{matrix} j\omega l_i + r_i + \dfrac{\rho_1 + \dfrac{1}{j\omega C_{op_1}}}{\mu_1 + 1} & \dfrac{-\rho_1}{\mu_1 + 1} & 0 \\ \dfrac{\dfrac{\mu_1}{j\omega C_{op_1}}}{\mu_1 + 1} & j\omega L_1 + \dfrac{\rho_1}{\mu_1 + 1} & -j\omega(L_1 + M) \\ 0 & -j\omega(L_1 + M) & j\omega(L_1 + L + 2M - \dfrac{1}{\omega^2 C_M} \\ 0 & j\omega M & -j\omega(L + M) \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{matrix} \right.$$

Right half of above———

$$\left. \begin{matrix} 0 & 0 & 0 \\ j\omega M & 0 & 0 \\ -j\omega(L + M) & 0 & 0 \\ j\omega L + r & 0 & 0 \\ 0 & \rho_2 + (j\omega l_o + r_o)(1 + j\omega C_{op_2} \rho_2) & 0 \\ -\rho_2 & \rho_2 + j\omega l_o + r_o & -\rho_2 \end{matrix} \right|$$

Let d_{i6} be the minor of the *i*th row and sixth column in either determinant Then

$$\bar{C} = \text{imaginary part of } \frac{d_{46} - (\mu_2 - j\omega C_{op_2}\,\rho_2)\,d_{56} + \left(\mu_2 + \frac{\rho_2}{R}\right)d_{66}}{-\,\omega\rho_2\,d_{66}}$$

$$= \text{imaginary part of } \frac{-\,d_{46} + (\mu_2 - j\omega C_{op_2}\,\rho_2)\,d_{56}}{\omega\rho_2\,d_{66}}$$

None of these terms are functions of R, so the important property of the circuit has been proved:

The resonance curve is symmetrical and the resonance capacity is independent of the shunt resistance R.

In the above it has been assumed that μ and ρ are constants, and that r_i, l_i, r_o and l_o do not depend on the voltage. These assumptions are only approximate, and it can be shown that in certain cases variation of these quantities may introduce errors in the results. To determine the magnitude of these errors the expression for $\bar{C}$ is worked out.

$$\bar{C} \doteqdot C_{op_2} \times \left(1 + \frac{\mu_2 r_o}{\rho_2}\right) - \text{imaginary part of } \frac{\delta_{44}}{\omega\delta}$$

in which

$$\delta = \begin{vmatrix} j\omega l_i + r_i + \dfrac{\rho_1 + \dfrac{1}{j\omega C_{op_1}}}{1 + \mu_1} & \dfrac{-\,\rho_1}{\mu_1 + 1} & 0 & 0 \\ j\omega l_i + r_i + \dfrac{1}{j\omega C_{op_1}} & j\omega L_1 & -\,j\omega(L_1 + M) & j\omega M \\ 0 & -j\omega(L_1 + M) & j\omega\left(L + L_1 + 2M - \dfrac{1}{\omega^2 C_M}\right) & -j\omega(L + M) \\ 0 & j\omega M & -\,j\omega(L + M) & j\omega L + r \end{vmatrix}$$

and δ_{44} is the minor of the term in the lower right corner.

Using the numerical values

$\dfrac{1}{\omega C_{op_1}} = 6 \times 10^6$	$j\omega l_i + r_i \doteqdot 10{,}000$ ohms (non-reactive)
$\rho = 10^6$	$j\omega L_1 = 500$ ohms
$\mu = 500$	$j\omega M = 100$ ohms

Thus $j\omega l_i + r_i$ is negligible in comparison with $\dfrac{1}{\omega C_{op_1}}$

$j\omega L_1$ is negligible in comparison with $\dfrac{1}{\omega C_{op_1}}$

If we neglect also C_M, we get simply

$$\bar{C} = \frac{1}{\left(\omega^2 L + \frac{r^2}{L}\right)} - C_{op_2}\left(1 + \frac{\mu_2 r_o}{\rho_2}\right)$$

2.32. *Construction.* The construction of the resonance apparatus is shown diagrammatically in Fig. 7. The oscillator and first amplifier are enclosed in separate copper cases, and the battery leads and batteries are also shielded. The coil in the output circuit of the amplifier is wound on the same form as the resonance coil L. The theory shows that this close coupling is not harmful. On the contrary, it makes shielding much simpler and permits the use of an oscillator of lower power than is generally used. The capacity of the resonance circuit is made up of four parts: two uncalibrated variable condensers, each 350 $\mu\mu$f. maximum, one cylindrical condenser 15 $\mu\mu$f. maximum and calibrated to read to 0.003 $\mu\mu$f., and the cell containing the electrolyte. This part of the apparatus was built into a rigid box of brass and the leads to and from it were fixed in tubular shields by means of supporting insulators.

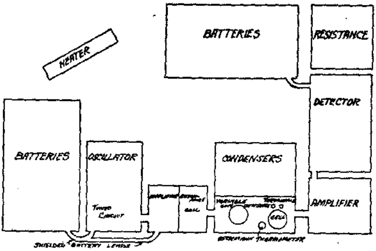

FIG. 7. *Resonance apparatus—arrangement of parts.*

In finding the resonance point, the condensers are adjusted so that the current in the galvanometer is zero for both positions of S (Fig. 5). The resonance capacity is then the average of the values when the switch is open and closed (since the curve has been shown to be symmetrical). The water in the cell is then replaced by solution and the small cylindrical condenser is varied until the "balance" is again obtained. The difference in capacity is then read on the dial of the small condenser. This method is much more sensitive than that of adjusting the condensers for maximum reading of the detector.

The amplifier and detector circuits were enclosed in separate shields and their batteries were shielded separately from those of the oscillator.

Voltmeters were permanently connected to the filament batteries and the rheostats kept adjusted to give the rated filament voltage. This was necessary since variations of filament currents produced small but noticeable errors in measurement. A change of 1% in voltage produced a capacity error less than 0.03 $\mu\mu$f.

3. Preliminary Tests and Discussion of Errors

3.1 *Bridge Method*

After a series of tests with the bridge described it became apparent that though the shielding had been very carefully constructed, coupling between various parts of the circuit still introduced errors for which no

correction could be made. The results of measurements on sodium chloride are roughly in accord with the resonance measurements given below, but are not considered sufficiently accurate to publish. The bridge is described, however, as it was found to be satisfactory for comparison measurements and might therefore be useful in later work.

3.2 *Resonance Method*

3.21 *Harmonics and frequency variations.* The theoretical discussion assumes a pure sine wave of constant frequency. A distorted wave form produces very small errors in resonance measurements with circuits having small dissipation. In the circuits used here, however, the effect of harmonics is much greater owing to the fact that the curves are so flat that the curves *for the harmonics overlap* on the curve for the fundamental. Fig. 8 illustrates the effect of harmonics. By inserting the tuned circuits (T_2 and T_3 in Fig. 5) and taking care not to overload valve V_2, the error due to harmonics is removed. The test for *symmetry* of the curve, given below, shows that this is realized experimentally.

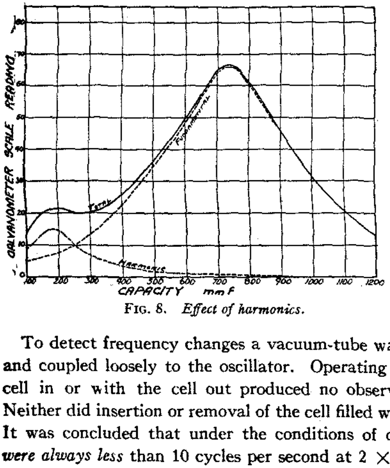

FIG. 8. *Effect of harmonics.*

To detect frequency changes a vacuum-tube wavemeter circuit was set up and coupled loosely to the oscillator. Operating switch S (Fig. 5) with the cell in or with the cell out produced no observable change of frequency. Neither did insertion or removal of the cell filled with either water or solution. It was concluded that under the conditions of operation *frequency changes were always less* than 10 cycles per second at 2×10^6 cycles per second.

Small variations in frequency occurred immediately after the currents had first been switched on, but ceased within an hour.

3.22 *Coupling of oscillator and detector.* Coupling between the detector and other circuits produces asymmetry of the resonance curve and hence errors in the results. This coupling was reduced to negligible magnitude by careful shielding. With the cell short-circuited no movement of the galvanometer could be detected when the generator was turned on and off. The symmetry of the resonance curve is additional evidence that this error has been removed.

Symmetry test. The capacity of a resistance of 180 ohms was measured for various values of C_1, the condenser in series with switch S (Fig. 5) corresponding to different heights on the curve. These measurements are given in Table I.

TABLE I

C_1, $\mu\mu$f.	Reading with resistance out	Reading with resistance in	Difference = parallel effective capacity of resistor	
350	13.2	20.6	−7.4	probable error
280	2.8	9.6	−6.8	= 0.2 div.
220	8.8	15.8	−7.0	= 0.06 $\mu\mu$f.
195	10.5	17.7	−7.2	
			Av. −7.1 units = 2.3 $\mu\mu$f.	

3.23 *Changes in effective tube capacities.* In section 2.31 the formula found for the resonance capacity is

$$\bar{C} \doteq \frac{1}{\left(\omega^2 L + \frac{r^2}{L}\right)} - C_{om}\left(1 + \frac{\mu_2 r_o}{\rho_2}\right)$$

The constants of the first valve do not occur in this approximate formula, so that their variations are unimportant. The constants of the second valve, however, are present in the formula and their effect may be regarded as a modification of the effective input capacity of the valve. The term r_o includes the impedance, Z, of the coupling unit between V_3 and V_4 in Fig. 5, and the input impedance of the next valve, V_4. The input impedance of this valve will depend on applied voltage, and therefore on R. For large values of R (*i.e.*, for dilute solutions), r_o will be reduced so that the reading for $\bar{C}$ will be too large. This was verified experimentally with an impedance Z equal to 5×10^5 ohms. When Z was reduced to its present value, 10^3 ohms, no error could be detected. In this case the correcting term cannot be greater than

$$C_{om}\left(1 + \frac{\mu_2 1000}{\rho_2}\right) = 0.015\left(1 + \frac{6 \times 10^6}{10^6}\right) \doteq 0.02\ \mu\mu f.,$$

which is less than 0.05% of the capacity of the smallest cell used.

It is for this reason that the valve V_3 is inserted in such a way as to have no value as an amplifier but merely serve to eliminate this error.

3.24 *Temperature variation.* Temperature variations may affect the results in two ways. The dielectric constant of electrolytes has a large temperature coefficient (0.4% per degree). Also the crystal frequency is dependent to a small extent on temperature. For these reasons the whole apparatus was enclosed in a box which could be kept at constant temperature. Temperature variations in the whole box were less than 0.1°. A Beckmann thermometer, immersed in the oil bath in which the cell was also placed, was used for measuring the cell temperature. All final readings were taken at 2.00 ± .01° on the Beckmann, corresponding approximately to 23° C.

3.3 *Errors Due to Cell Construction*

A number of errors may occur if suitable precautions are not taken in cell construction. These are common to the cells used in both methods, except that in the three-electrode cells used with the bridge no errors due to insulators present in the field can occur.

3.31 *Inductance of cell leads.* Reference to Fig. 9 shows that the true capacity of the cell bears the following relation to the measured capacity:—

$$C_{eff} \doteq C\left(1 + \omega^2 LC - \frac{Lg^2}{C}\right)$$

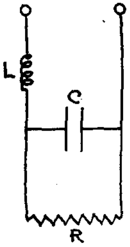

FIG. 9. *Circuit equivalent to cell and leads for inductance correction.*

where L is the inductance of leads, and g, the conductance of cell. Terms in L^2 are neglected.

This formula for inductance error has also been derived by Lattey, but the cells he used were so small that the correction was unimportant in his work. The use of small cells, however, greatly decreases the sensitivity. Jezewski (9) neglects the second term, $\frac{Lg^2}{C}$, which may have influenced his results as shown below. Most other observers have neglected both terms. The importance of the correction is illustrated below.

For a loop of wire 1 cm. in radius and made of wire of 1 mm. diameter the approximate inductance is 5×10^{-8} henries. It is very difficult to make cell leads with lower inductance than this. For this value and a cell, of 10 $\mu\mu$f capacity when full of water, containing $N/100$ sodium chloride at two megacycles' frequency,

$$C = 10(1 + (5 \times 10^{-6}) - 0.01)$$

For this case the term $\omega^2 LC$ is obviously negligible, whereas the term $\frac{Lg^2}{C}$ is 1%. The first term becomes more conspicuous at higher frequencies and both terms become larger for cells of larger capacity.

Several experimental tests were made which confirmed the presence of errors due to inductance. In the case of one cell, the impedance was measured with the cell short-circuited and was found to be about one ohm, corresponding to $L = 10^{-7}$. The impedance was reduced by placing a 0.05 $\mu\mu$f. condenser in series with the leads, indicating that the impedance had been inductive. This agrees in magnitude with the values of L found below. However, no direct method is sufficiently sensitive to evaluate so small an inductance, so the indirect method described below must be used.

3.32 *Insulators in the solution.* Jezewski points out that when the insulators supporting the electrodes project into the solution the apparent capacity of the cell is increased when the concentration is increased. The same effect is produced by dirt on the electrodes. A small spot of shellac put on one electrode was shown to produce errors as large as 50% for fairly concentrated solutions. In designing the cells used here care was taken to avoid these errors. The check obtained with two entirely different types of cell shows that this and similar errors have been eliminated.

3.4 *Corrections.* The correction for inductance was made as follows. Cell B shown in Fig. 4B was constructed with movable plates so that the capacity could be changed without greatly varying the inductance. If readings are

taken with such a cell for various capacity settings, C_1, the measured capacity for a given solution is

$$C = C_o\,(\epsilon + \Delta\epsilon) - LG^2 = C_o\,(\epsilon + \Delta\epsilon) - a\gamma^2 C_o^2$$

where γ is the conductivity of the solution and G is the conductance of the cell.

Then

$$\frac{C - \epsilon C_o}{\epsilon C_o} = \frac{\Delta\epsilon}{\epsilon} - a\gamma^2 C_o$$

The term on the left is the measured difference between the capacity of the cell when it is filled with solution and that when it is filled with water. If this measured percentage change is plotted in a graph against C_o the curves should be a family of straight lines, one for each concentration. The slopes of the lines should be proportional to the inductance and to the square of the conductivity. The intercepts on the axis of ordinates give the corrected percentage change in dielectric constant of each solution.

Similarly the cell C (Fig. 4C) may be used with the plates separated by different distances. The inductance is not greatly changed in this way although the capacity may be changed by a factor of two. The level of the solution in this cell is not as important as it is in the silver cell, so that more accurate readings can be made. The level of the liquid in both cells was kept constant by filling with a measured volume of solution. The results of the measurements are given in the next section.

4. Measurements

4.1 *Absolute measurements on sodium chloride.* A silver-plated brass cell was constructed in such a way that its capacity could be varied (Fig. 4B). This cell was used to determine the inductance correction. Table II gives the averages of three sets of readings made with this cell. The capacity, C_o,

TABLE II

AVERAGE READINGS WITH CELL B (SILVER PLATED)

Concentration, millinormal	ΔC	G	$\frac{\Delta C}{G}$	ΔC	G	$\frac{\Delta C}{G}$
	C_o = 740 units			C_o = 545 units		
0.5	1.3	1.78	0.8	2.8	1.31	2.1
1.0	1.0	3.54	0.3	2.4	2.61	0.9
2.0	−7.5	7.0	−1.1	−2.0	5.18	−0.4
3.0				−11.5	7.7	−1.5
	C_o = 462 units			C_o = 393 units		
1.0	3.0	2.21	1.4	2.2	1.88	1.2
2.0	0.9	4.39	0.2	1.7	3.73	0.4
3.0	−2.2	6.5	−0.3	−0.7	5.5	−0.1

of the cell when filled with water is corrected for the capacity of the leads by calibrating with benzene. The units in which it is expressed are scale divisions of the variable condenser.

$$1\ \mu\mu f. = 3.20 \text{ units.}$$

The column marked ΔC gives the apparent increase in capacity when water is replaced by the solution. The cell conductance, G, is calculated from tabulated conductivities and agrees within 5% with the value measured on a commutated-direct-current bridge. It is used in making the inductance correction.

All measurements were made at 23.0° C. and 2×10^6 cycles per second. Assuming as above, $\Delta C = \frac{\Delta\epsilon}{\epsilon} C_o - LG^2$ (Section 3.4), and $C_o = AG$, where A is a constant for a given solution, $\frac{\Delta C}{G} = A \frac{\Delta\epsilon}{\epsilon} - LG$.

The data in Table I are plotted in Fig. 10. For each solution there is no systematic departure from linearity in the curves for $\frac{\Delta C}{G}$ against G. The slope of the curve gives the lead inductance, L. It is to be noted that any error other than one of the form (constant $\times$ G), would be detected because it would produce curvature in the lines in this graph. There is no reason to expect an error of the form (constant $\times$ G). The lines for each concentration should be parallel but not necessarily coincident. The points for 1.0 and 2.0 millinormal concentrations are the most reliable and only these were used.

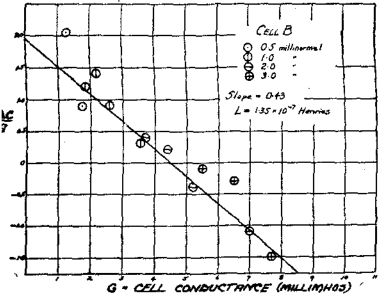

FIG. 10. *Determination of correction for cell lead inductance.*

Using the value of L (1.35×10^{-7} henries) determined from Fig. 10, the corrected values for $\frac{\Delta\epsilon}{\epsilon} C_o$ are found from the formula. Dividing by the capacity of the cell the following values for the percentage increase in dielectric constant are found.

TABLE III

PERCENTAGE INCREASE IN DIELECTRIC CONSTANT

(Sodium chloride in Cell B)

Concentration	C_o = 740	C_o = 545	C_o = 462	C_o = 393	Average
0.5	0.4	0.6			0.5
1.0	0.9	1.0	1.1	0.9	1.0
2.0	1.8	1.7	2.0	1.9	1.95
3.0		2.9	2.5	3.0	2.8

In the same way readings were taken with Cell C for two capacities.

TABLE IV

READINGS WITH CELL C (PLATINUM-GLASS)

Concentration, millinormal	$C = 398$			$C = 153$		
	ΔC	G, micromhos	$\frac{\Delta C}{G}$	ΔC	G, micromhos	$\frac{\Delta C}{G}$
0.6	2.8	1.15	2.43	1.5	0.44	3.40
1.0	3.6	1.91	1.90	2.0	0.73	2.74
1.5	3.2	2.85	1.14			
2.0	3.4	3.78	0.90	3.1	1.45	2.14
3.0	− 1.0	5.64	−0.17	3.15	2.16	1.46
4.0	− 5.7	7.5	−0.76	3.5	2.88	1.21
5.0	−13.0	9.2	−1.7	2.4	3.59	0.67
6.0				1.5	4.30	0.35
8.0				−1.9	5.65	−0.35
10.0				−7.0	7.00	−1.0

The values of L calculated from the data for each concentration which was measured in both cells are shown in Table V.

TABLE V

Concentration	2	3	4	5	Average
L, henries	1.6	1.4	1.3	1.4	1.42×10^{-7}

Using this value of L the corrected readings are as shown in Table VI.

TABLE VI

PERCENTAGES INCREASE IN DIELECTRIC CONSTANT

(Sodium chloride in Cell C)

Concentration, millinormal	$C_0 = 398$	$C_0 = 153$	Average	Concentration, millinormal	$C_0 = 398$	$C_0 = 153$	Average
0	0	0	0	2.0	2.5	2.6	2.55
0.2	0.3		0.3	3.0	3.3	3.4	3.35
0.4	0.6		0.6	4.0	4.8	4.7	4.75
0.6	0.8	0.8	0.8	5.0	5.5	5.3	5.4
0.8	1.1		1.1	6.0		6.5	6.5
1.0	1.3	1.4	1.35	8.0		8.0	8.0
1.5	1.7		1.7	10.0		9.8	9.8

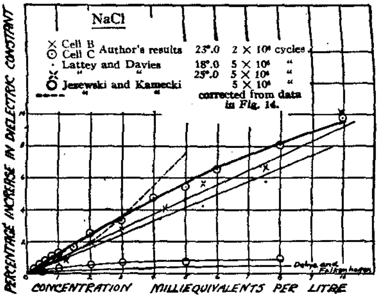

FIG. 11. *Dielectric constant of sodium chloride solutions.*

These values are plotted in Fig. 11.

4.2 *Comparison measurements on several electrolytes.* The errors for which corrections were made in the last section are functions of cell conductance only. Different electrolytic solutions of the same conductivity can therefore be compared without any corrections. This was done with several solutions to test the method. The high-frequency conductivity of the solutions used does not greatly differ from the d–c. conductivity, so the solutions could be matched on a commutated d–c. bridge. The value of R, the compensating resistance in the plate circuit of the detector, is a measure of the conductivity. As a check, the readings of this resistance were recorded and showed that when the d–c. conductivities were made equal the high-frequency conductivity did not differ by more than 2%. The values of ΔC here are the differences

TABLE VII

Substance	Solutions matched with 0.001 N common salt			Solutions matched with 0.002 N common salt			
	Conc., milli-normal	$\frac{\Delta C}{C_o}$, %, $C_o = 398$	$\frac{\Delta \epsilon}{\epsilon}$, %	Conc., milli-normal	$\frac{\Delta C}{C_o}$, %, $C_o = 398$	$\frac{\Delta C}{C_o}$, %, $C_o = 153$	$\frac{\Delta \epsilon}{\epsilon}$, %
NaCl	1.00	—	1.2	2.00	—	—	2.3
HCl	.29	−0.05	1.2	.55	0.05	0.1	2.4
K_2SO_4	.82	.15	1.3	1.70	0.22	0.4	2.6
$CuSO_4$	1.10	0.8	2.0	2.35	0.9	0.8	3.2
$MgSO_4$	1.04	0.8	2.0	2.10	1.0	1.1	3.3
$K_4Fe(CN)_6$	1.05	0.3	1.5	2.15	0.6	—	2.9

Substance	Solutions matched with 0.005 N common salt			Solutions matched with 0.010 N common salt		
	Conc., milli-normal	$\frac{\Delta C}{C_o}$, %, $C_o = 153$	$\frac{\Delta \epsilon}{\epsilon}$, %	Conc., milli-normal	$\frac{\Delta C}{C_o}$, %, $C_o = 153$	$\frac{\Delta \epsilon}{\epsilon}$, %
NaCl	5.00	—	5.7	10.00	—	9.5
HCl	1.40	0	5.7	2.76	0.5	10.0
K_2SO_4	4.3	0.4	6.1	8.75	1.4	10.9
$CuSO_4$	7.0	1.5	7.2	16.7	3.0	12.5
$MgSO_4$	6.3	1.5	7.2	13.4	2.7	12.2

TABLE VIII

SUMMARY—% INCREASE IN DIELECTRIC CONSTANT

Conductivity $\times 10^{-3}$	NaCl	HCl	K_2SO_4	$CuSO_4$	$MgSO_4$	$K_4Fe(CN)_6$
1.07	1.2	1.2	1.3	2.0	2.0	1.5
2.10	2.3	2.4	2.6	3.2	3.3	2.9
5.20	5.7	5.7	6.1	7.2	7.2	
9.80	9.5	10.0	10.9	12.5	12.2	

between the capacity with the solution in the cell and that with common salt of the same conductivity. $\frac{\Delta\epsilon}{\epsilon}$ is then calculated by adding values from the graph for sodium chloride (Fig. 11.).

These results are plotted in Fig. 12 with conductivity as abscissa and in Fig. 13 with concentration as abscissa.

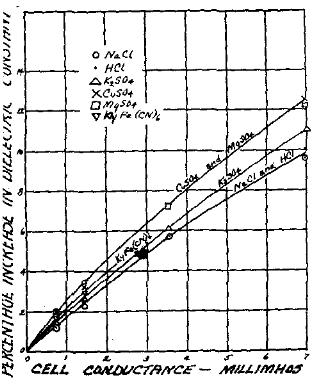

FIG. 12. *Dielectric constant of electrolytes plotted against cell conductance.*

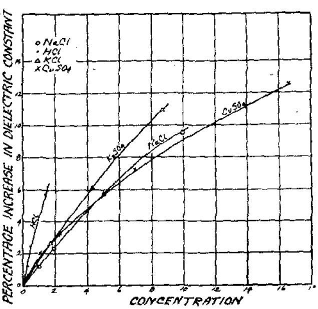

FIG. 13. *Dielectric constant of electrolytes plotted against concentration.*

5. Discussion of Results

5.1 The work described here differs in several respects from previous work with resonance methods. The method of finding the resonance point as described in Section 2.32 is not new but has not been used before in work on electrolytes. As is well known, it is possible by this method to increase the sensitivity greatly, for it is possible to adjust the size of the condensers so that the setting is made at the steepest part of the resonance curve instead of at the flat peak. This method is possible only when the resonance curve is symmetrical. It has been shown both theoretically and experimentally that

his is so. The circuit calculations, in addition to proving the symmetry of he curve, show that errors due to coupling are absent, thus permitting the ıse of a driving coil very close to the resonance coil. The power required n the oscillator is thereby decreased, thus greatly facilitating the shielding ›f the circuit. The shielding was such that the measurements were quite ınaffected by the movements of the observer.

The use of a quartz oscillator and of tuned filter circuits (T_2 and T_3, Fig. 5) has been shown to give constant frequency and pure harmonic wave form. The necessity for these has been recognized before but the means of attaining them have not been used. Jezewski, however, corrected for errors due to frequency changes by comparison with a subsidiary quartz oscillator.

The use of two cells of very different construction, both of which have several values of capacity, made possible the evaluation of error due to inductance of cell leads, and also assured the absence of other errors due to cell construction.

5.2 *Accuracy.* The accuracy with which readings may be repeated is better than $\pm$ 0.1% for solutions up to 3 millinormal and $\pm$0.5% for the strongest solutions measured. The accuracy of the final results, however, is not as great since the correction for inductance of the cell leads is large and comparatively difficult to estimate. From the agreement obtained with the two cells, it seems likely that the results up to 3 millinormal are correct within $\pm$0.5%. For the strongest solutions the error may be as large as $\pm$2%.

5.3 *Comparison with results of other observers and with theory.* Debye and Falkenhagen (2) predict an increase in dielectric constant with increasing concentration due to the "ionic atmosphere." This effect decreases with increasing frequency. Tables giving the predicted values in special cases are given by Falkenhagen and Vernon (5). As stated in the introduction, the experimental results of many observers for very high frequencies are in agreement with the theory.

For lower frequencies, however, the agreement holds only in the case of Jezewski's (9) work. As shown in Fig. 11, the author's results, which agree approximately with those of Lattey and Davies (10, 11), show an increase in dielectric constant much larger than the predicted values for sodium chloride. For other salts the difference from the theory is of the same order.

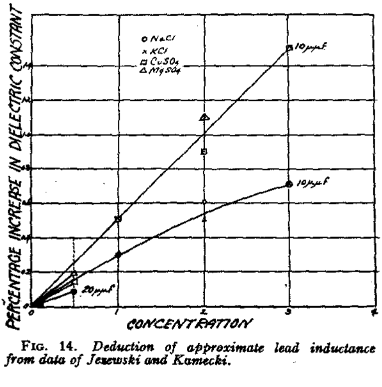

FIG. 14. *Deduction of approximate lead inductance from data of Jezewski and Kamecki.*

The apparent agreement of Jezewski with theory seems to be accidental, as he has not properly corrected for the inductance of cell leads. In Table III on page 565, reference (9), Jezewski gives results for several salts. The results for concentration 0.0005 N were obtained with a cell of 20 $\mu\mu$f. capacity, while the remainder were obtained with a smaller cell of 10 $\mu\mu$f. capacity. Fig. 14, which is drawn from Jezewski's table, shows a definite difference between the results with the large cell and the curve for the small cell. If, as seems likely, this difference is due to inductance, an approximate evaluation of the error can be made by the method outlined above (Section 3.4). This was done on the assumption that the inductance was the same for both cells. As this may not be so, the correction is not to be relied on, but is given merely to show a possible explanation for the discrepancy between the results of Jezewski and those of Lattey and of the author.

The "corrected" values for sodium chloride, from Jezewski's work, are plotted in Fig. 11.

No attempt is made to account for the disagreement with theory as more data would first be necessary. It is hoped that these measurements will before long be extended to higher frequencies and to a wider range of salts.

Acknowledgment

The author wishes to acknowledge his indebtedness to Prof. E. F. Burton whose interest in the work has been unfailing, and to Mr. Arnold Pitt and Dr. Alan Young, for many helpful suggestions during the course of this research.

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THE TRANSMISSION OF SOUND BY A SERIES OF EQUIDISTANT PARTITIONS[1]

By Donald G. Hurst[2]

Abstract

The transmission of normally incident sound waves through a series of similar, equally spaced partitions has been calculated for the cases of infinite partitions and of circular partitions. The transmission characteristics depend on the solution of simultaneous linear difference equations for the velocity potentials. The array of partitions possesses interesting properties as a filter, and to illustrate these the change in intensity levels due to glass partitions is calculated numerically for 1, 2, 5, and 10 partitions.

Introduction

Problems of acoustic insulation, to which much attention is now being given (8), suggest the calculation of transmission through a number of partitions of building material of various types. The question has recently been made the subject of an investigation by Constable at the National Physical Laboratory (2).

The theoretical treatment in this paper is concerned principally with the transmission of normally incident sound waves through a number of equally spaced, infinite, rigid partitions of the same mass per unit area. In these circumstances the velocity potentials in the air spaces may be determined as solutions of simultaneous linear difference equations arising from the boundary conditions at each partition.

This simplified problem shows that the array of partitions has interesting characteristics as an acoustic filter, *i.e.*, the arrangement is selective, readily transmitting certain frequency bands of the acoustic spectrum while remaining opaque to others. A numerical case has been worked out completely for systems of 1, 2, 5 and 10 glass partitions of mass 0.72 gm./cm.^{2} and spacing 5 cm. The regions of attenuation have also been found for a semi-infinite sequence of cellophane partitions of mass 0.0036 gm./cm.^{2} and spacing 5 cm.

The general procedure of this paper might be extended to equally spaced partitions through each of which the propagation of sound is taken into account. If desired, the dissipation of energy in each plate may be taken into account as well.

When the partitions are considered to be of finite size and inserted in a rigid wall, the transmission characteristics are affected by diffraction phenomena at the first and last partition. If we consider each partition to be a rigid circular piston the problem is still capable of rigorous solution in terms of cylindrical wave functions, making use of Hankel's inversion theorem in the manner employed in a recent paper by King (6). It is beyond the scope of the present paper to consider any except rigid partitions, so that no allowance is made for other possible modes of vibration of actual panels.

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Contribution from the Department of Physics, McGill University, Montreal, Canada.
[2] *Holder of a bursary under the National Research Council of Canada.*

When the incident wave is inclined to the first partition, the latter does not move if it is *infinite* and *rigid*, since the resultant pressure variation on it is negligible in comparison with its mass. However, for finite panels we should expect a wave slightly inclined to the normal to act similarly to a normally inclined wave. The problem may be stated for circular partitions capable of oscillating under specified constraints, and the solution then depends on the corresponding problem of oblique diffraction by a piston surrounded by an infinite, rigid flange. Such problems are extremely difficult and are beyond the scope of the present paper.

Infinite, Plane, Rigid Partitions

(a). Let us consider the case of p equally spaced rigid plates or partitions of mass m_0 per unit area at a distance b apart (Fig. 1).

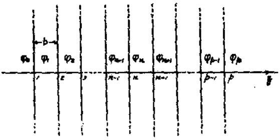

FIG. 1. *Series of plane, infinite, rigid partitions.*

A plane wave is normally incident on the first. The velocity potential φ, apart from the time factor $e^{i\omega t}$ which will be omitted for purposes of brevity, is determined from the equation

$$\frac{d^2\varphi}{dz^2} = -\frac{\omega^2}{c^2}\,\varphi = -\kappa^2\varphi\,, \tag{1}$$

where c is the velocity of sound in air, $\omega = 2\,\pi f$ where f is the frequency, $\kappa = \omega/c$ and z is measured normally to the plates in the direction of propagation of the incident wave. In the nth interspace the appropriate solution of Equation (1) is

$$\varphi_n = A_n e^{-i\kappa(z-nb)} + B_n e^{i\kappa(z-nb)}\,. \tag{2}$$

The first term corresponds to the forward wave, the second to the reflected wave.

The particle velocity is given by

$$\dot{\xi} = -(\partial\varphi/\partial z)\,, \tag{3}$$

where ξ is the displacement and $\dot{\xi}$ as usual denotes $\partial\xi/\partial t$. At the $(n+1)^{th}$ partition, continuity of velocity requires

$$\dot{\xi}_{z=nb} = -(\partial\varphi_n/\partial z)_{z=nb} = -(\partial\varphi_{n+1}/\partial z)_{z=nb}\,. \tag{4}$$

The variation of pressure is given by

$$\delta p = \rho_0\dot{\varphi}\,, \tag{5}$$

where ρ_0 is the mean density of the air.

It may be supposed that each partition is able to perform free oscillations of a simple harmonic type under an elastic restoring force $\mu\xi$ per unit area, so that the dynamical equation for the $(n+1)^{th}$ partition is

$$(\delta p_n)_{z=nb} - (\delta p_{n+1})_{z=nb} = (m_0\ddot{\xi} + \mu\xi)_{z=nb}\,.$$

For periodic oscillations, $\ddot{\xi} = -\omega^2\xi$, so that Equation (5) gives

$$\rho_o (\dot{\varphi}_n - \dot{\varphi}_{n+1})_{z=nb} = m_o (1 - \omega_o^2/\omega^2)\ddot{\xi}_{z=nb} ,$$

and consequently

$$\dot{\xi}_{z=nb} = \frac{(\varphi_n - \varphi_{n+1})_{z=nb}}{(m_o/\rho_o)(1 - \omega_o^2/\omega^2)} = -\left(\frac{\partial \varphi_n}{\partial z}\right)_{z=nb} = -\left(\frac{\partial \varphi_{n+1}}{\partial z}\right)_{z=nb}. \tag{6}$$

where $\omega/2\pi$ is the impressed frequency, and $\omega_o/2\pi$ is the natural frequency of the partitions. From Equations (2) and (6) is derived the expression

$$\frac{A_n + B_n - A_{n+1}e^{i\kappa b} - B_{n+1}e^{-i\kappa b}}{(m_o/\rho_o)(1 - \omega_o^2/\omega^2)} = i\kappa(A_n - B_n) = i\kappa(A_{n+1}e^{i\kappa b} - B_{n+1}e^{-i\kappa b}).$$

On writing

$$\gamma = e^{i\kappa b} ;\ \nu = \tfrac{1}{2}(\kappa m_o/\rho_o)(1 - \omega_o^2/\omega^2) \tag{7}$$

we have for the A's and B's the simultaneous difference equations

$$A_n - A_{n+1}\gamma = B_n - B_{n+1}\gamma^{-1} \tag{8}$$

and

$$A_n(1 - i\nu) - A_{n+1}\gamma = -i\nu B_n . \tag{9}$$

By elimination of B_n, there is obtained for A_n the linear difference equation

$$A_{n+1} - 2A_n \cosh \alpha + A_{n-1} = 0,$$

where

$$\cosh \alpha = \cos \kappa b - \nu \sin \kappa b. \tag{10}$$

It is easily seen that the solution may be written

$$A_n = A \sinh (n\alpha + \epsilon_1) = A'e^{n\alpha} + A''e^{-n\alpha}. \tag{11}$$

Similarly

$$B_n = B \sinh (n\alpha + \epsilon_2) = B'e^{n\alpha} + B''e^{-n\alpha}, \tag{12}$$

where A, A', A'', B, B', B'', ϵ_1, and ϵ_2 are independent of n. That value of α which is positive, or whose real part is positive, is chosen.

(b). When the number of partitions is infinite it is seen that the appropriate solutions are

$$A_n = A''e^{-n\alpha} ;\ B_n = B''e^{-n\alpha} ,$$

and hence there will be attenuation of amplitude if α has a real part, a condition which requires that

(i) $\cosh \alpha > 1$ or (ii) $\cosh \alpha < -1$.

If we define $u = \frac{1}{2}\kappa b$, the first condition becomes

$$\sin 2u\ (\tan u + \nu) < 0.$$

Thus if

$$\left.\begin{array}{l}\sin 2u > 0, \text{ we must have } \tan u < -\nu \\ \sin 2u < 0, \text{ we must have } \tan u > -\nu\end{array}\right\}. \tag{13}$$

The second condition becomes

$$\sin 2u\ (\cot u - \nu) < 0.$$

Thus if

$$\left.\begin{array}{l}\sin 2u > 0, \text{ we must have } \cot u < \nu \\ \sin 2u < 0, \text{ we must have } \cot u > \nu\end{array}\right\} \quad (14)$$

In studying Equations (13) and (14) as conditions of attenuation of amplitude, it will be sufficient as a preliminary step to neglect the effect of the natural frequency of the partitions and to write $\omega_o = 0$. This makes

$$\nu = \tfrac{1}{2}\kappa m_o/\rho_o = m_o u/(b\rho_o) = \eta u,$$

where

$$\eta = m_o/(b\rho_o).$$

Since $\sin 2u = 2 \tan u \cos^2 u$, $\sin 2u$ and $\tan u$ have the same sign, and since ηu is always positive we obtain from Equations (13) and (14)

$$\left.\begin{array}{l}0 > \tan u > -\eta u \\ 0 < \cot u < \eta u\end{array}\right\}(\omega_o = 0) \quad (15)$$

as the possible conditions. These conditions are illustrated graphically in Figs. 2 and 3. It will be noticed that the transmission bands become successively narrower with increasing frequency.

The effect of the term $1 - \omega_o^2/\omega^2$ is shown by the Curves (ii) of both figures which represent $y = \mp\nu$ respectively. For $\omega \gg \omega_o$ there is a small reduction in the width of the attenuation bands. However, for $\omega \leqslant \omega_o$ the effect may be considerable, and as is seen in Fig. 2, a band of attenuation corresponding to the condition $0 < \tan u < -\nu$ is introduced near the origin. Inasmuch, however, as the natural frequency of an actual panel is usually low, relative to audible frequencies (if we neglect possible radial and transverse modes of vibration), the disturbance due to ω_o does not extend appreciably into the audible frequencies.

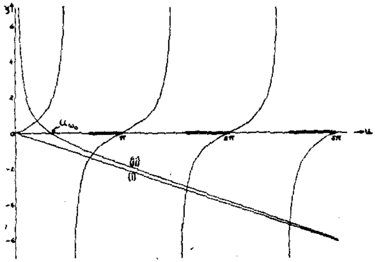

FIG. 2. *Tan u plotted against u; (i) is* $y = -\frac{2}{\pi}u$ $\left(\eta = \frac{2}{\pi}\right)$. *The regions of attenuation are marked by the heavy lines on the axis of u. They are contained between the roots of* $\tan u = -\frac{2}{\pi}u$ *and* $u = \pi, 2\pi, 3\pi \ldots$.

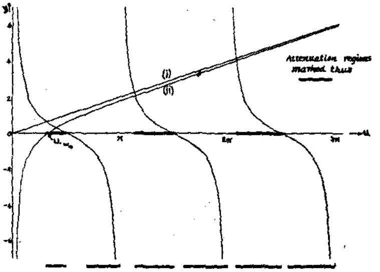

FIG. 3. *Cot u plotted against u;* (i) *is* $y = \frac{2}{\pi}u$. *Here the regions of attenuation are bounded by the roots of* $\cot u = \frac{2}{\pi}u$ *and* $u = \frac{1}{2}\pi, \frac{3}{2}\pi, \ldots$ *The combined system of attenuation bands is shown at the bottom of the figure.*

Transmission Characteristics of a Finite Number of Partitions

The existence of alternate regions of attenuation and perfect transmission in the semi-infinite case leads one to expect wide variations in the characteristics of a finite number, p, of partitions.

The sound wave generated by the last partition is a progressive wave traveling in the direction of the incident wave, and it will be supposed that it is not reflected, so that, referring to Equation (2), $B_p = 0$. According to Equation (12) therefore

$$B_n = -B \sinh (p - n)\alpha. \tag{16}$$

On giving n the value $n = p-1$ in the difference equations (8) and (9), and remembering that $B_p = 0$,

$$A_{p-1} - \gamma A_p = B_{p-1} = -(i\nu)^{-1} \{A_{p-1}(1 - i\nu) - \gamma A_p\}$$

is derived.

Let $\beta = p\alpha + \epsilon_1$. Then from the above equation

$$\cosh \beta = \cos \kappa b + (1/\nu) \sin \kappa b \tag{17}$$

and

$$\sinh \beta = (i/\nu) \sinh \alpha. \tag{18}$$

Finally

$$\frac{A_p}{A_o} = \frac{\sinh (p\alpha + \epsilon_1)}{\sinh \epsilon_1} = \frac{\sinh \beta}{\sinh (\beta - p\alpha)}. \tag{19}$$

The intensity level of the incident sound (1) is

$$\alpha_o = 10 \log_{10} (I_o/10^{-16}) \text{ decibels,}$$

where $I_o/10^{-16}$ is the ratio of the incident sound intensity in watts/cm^2 to the standard reference intensity (10^{-16} watt/cm^2). Similarly the intensity level of the emerging sound is

$$\alpha_p = 10 \log_{10} (I_p/10^{-16}) \text{ db.}$$

The difference in levels due to the array of partitions will be denoted by δ_{DB}, and is given by

$$\delta_{DB} = \alpha_o - \alpha_p = 10 \log_{10} (I_o/I_p).$$

Since the waves are plane,

$$(I_o/I_p) = |A_o/A_p|^2,$$

so that

$$\begin{aligned}\delta_{DB} &= 20 \log_{10} |A_o/A_p| \\ &= 20 \log_{10} \left|\frac{\sinh (\beta - p\alpha)}{\sinh \beta}\right|.\end{aligned} \tag{20}$$

It may be shown, as indeed is to be expected from energy considerations, that

$$|A_o|^2 = |B_o|^2 + |A_p|^2$$

i.e., the intensity of the incident wave is equal to the sum of the intensities of the reflected wave and the transmitted wave.

Single and double partitions, corresponding to $p = 1$ and $p = 2$, have been examined theoretically and experimentally in recent papers by Davis (3) and Constable (2). The formulas obtained, transformed to the present notation are

$$|A_0/A_1|^2 = 1 + \nu^2 \quad \text{(single panel)}$$

and

$$|A_0/A_2|^2 = 1 + 4\nu^2 \cosh^2 \alpha \quad \text{(two equal panels)}$$

to which Equation (19) reduces for the values $p = 1$ and $p = 2$.

Numerical Calculation

To illustrate the results of the preceding sections some numerical examples may be considered. We take as partitions panes of glass for which $m_o = 0.72$ gm./cm.², and which are separated by a distance $b = 5$ cm. At a temperature of 20° C. and normal pressure, $\rho_o = 1.20 \times 10^{-3}$ gm./cm³, $c = 3.44 \times 10^4$ cm./sec.

Hence $\eta = m_o/(b\rho_o) = 120$. The natural frequency is taken as 18.3 cycles per second, thus making $\nu = 300\kappa - (300\kappa)^{-1}$ and greatly simplifying the arithmetic.

The method of computation is to choose κ, then compute ν, $\cos \kappa b$ and $\sin \kappa b$, and finally $\cosh \alpha$, $\cosh \beta$. From these α and β are readily found. With the aid of Kennelly's chart (5), it is a simple matter to obtain $|\sinh \beta|$ and $|\sinh(\beta - p\alpha)|$. One calculation of α and β suffices for any number of partitions. δ_{DB} is calculated from Equation (20).

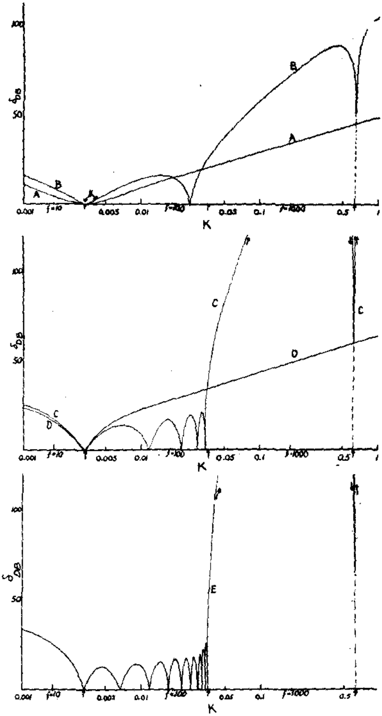

FIG. 4. *Reductions in intensity levels (measured in decibels) due to various numbers of partitions, plotted against a logarithmic scale of κ ($=2\pi f/c$). The standard partition is taken as glass, having a mass per unit area $m_o = 0.72$ gm./cm.² and natural frequency $f_o = 18.3$ cycles per second. The spacing between partitions is 5 cm.*

The separate curves are: A, one partition; B, two partitions; C, five partitions; D, one partition having $m_o = 5 \times 0.72$ gm./cm.² and $f_o = 18.3$ cycles per second; i.e., its weight equals the combined weight of the five partitions in C; E, ten partitions. The transition to the semi-infinite case can be seen taking place as the number of partitions is increased. In all curves the first zero is at the resonant frequency of a single partition; the other zeros ($p - 1$ in number in the first transmission region) occur at the points where $p\alpha = i\pi \times$ (integer) and $\beta \neq 0$, since then $|A_o/A_p| = 1$ and $\delta_{DB} = 0$.

The computations were performed for 1, 2, 5, and 10 partitions and for a single partition of mass per unit area equal to that of the five partitions combined. The results are plotted in Fig. 4.

Although the higher frequency ends of the attenuation bands are determined solely by the spacing, the width of the bands decreases with decreasing weight of the partition. The following table gives a comparison for the glass partitions considered above, and similar partitions (*e.g.*, of cellophane) of mass per unit area $m_o = 3.6 \times 10^{-3}$ gm./cm.^{2} so that $\eta = 0.60$. Using Equation (15) it is found (4, pp. 36 and 37) that the regions of attenuation are given by the data in Table I. Thus, while with glass the regions of transmission are extremely narrow, they are much wider with cellophane and might possibly be observed experimentally. Owing to the smaller values of ν for cellophane it is to be expected that the reduction will not be as great as for the heavier partitions. As an example, the reductions for glass and cellophane at 3300 cycles per second are compared (Table II).

TABLE I

REGIONS OF ATTENUATION

Glass	Cellophane
$200 < f < 3440$ $3446 < f$ Further transmission regions are too narrow to be indicated and all frequencies above 200 can be considered as attenuated.	$2230 < f < 3440$ $4850 < f < 6880$ $7840 < f < 10{,}320$ $11{,}000 < f < 13{,}700$ etc.

TABLE II

Number of partitions	δ_{DB} (f = 3300 cycles per second)	
	Glass	Cellophane
1	45	2.6
2	78	7.2
5	Too large to have practical significance	21
10		44

It should however be recalled that 10 partitions of the cellophane have a mass only 0.05 times that of a single pane of glass.

Transmission Through a Series of Circular Panels Set Into a Rigid Wall, Taking into Account Diffraction Effects

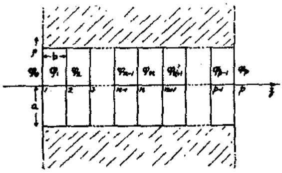

FIG. 5. *Cross section of series of circular panels set into a rigid wall.*

The cross section of the series of panels is shown in Fig. 5.

In terms of cylindrical co-ordinates (ρ, z) the velocity potential may be written (6) in the form

$$\varphi_o = 2A_o \cos \kappa z + \int_0^\infty f_o(\lambda) e^{\mu z} \left(\frac{\lambda}{\mu}\right) J_o(\lambda\rho)\, d\lambda\,, \tag{21}$$

where $\mu^2 = \lambda^2 - \kappa^2$, and z is measured from the first plate in the direction of propagation of the incident wave. The first term on the right represents a plane

wave which undergoes perfect reflection at the wall, and the second term is a solution of the wave equation representing reflection by the circular panel. The function $f_o(\lambda)$ is determined by the boundary condition

$$-\left(\frac{\partial \varphi_o}{\partial z}\right)_{z=0} = \begin{cases} \dot{\xi}_o, & 0 < \rho < a \\ 0, & a < \rho < \infty \end{cases} \tag{22}$$

and $\dot{\xi}_o$ is constant since the partitions are rigid.

$$\left(\frac{\partial \varphi_o}{\partial z}\right)_{z=0} = \int_0^\infty f_o(\lambda)\lambda J_o(\lambda\rho)d\lambda\,.$$

According to Hankel's inversion theorem, if

$$F(\rho) = \int_0^\infty f(\lambda)\lambda J_o(\lambda\rho)d\lambda$$

then

$$f(\lambda) = \int_0^\infty F(\rho)\rho J_o(\lambda\rho)d\rho\,.$$

In the present instance

$$f_o(\lambda) = \int_o^a (-\dot{\xi}_o)\,\rho\, J_o(\lambda\rho)\, d\rho = -\dot{\xi}_o\left(\frac{a}{\lambda}\right) J_1(\lambda a)\,, \tag{23}$$

so that

$$\varphi_o = 2A_o\cos\kappa z - a\dot{\xi}_o\int_0^\infty e^{\mu z}\, J_o(\lambda\rho)\; J_1(\lambda a)\,\frac{d\lambda}{\mu}. \tag{24}$$

The dynamical equation for the first partition is

$$\int_o^a (\delta p_o - \delta p_1)_{z=0}\, 2\pi\rho d\rho = \pi a^2 m_o\,(1-\omega_o^2/\omega^2)\,\ddot{\xi}_o\,.$$

Equations (5) and (24) give

$$A_o - (\dot{\xi}_o/a)\int_o^a \rho d\rho\int_o^\infty J_o(\lambda\rho)\, J_1(\lambda a)\,\frac{d\lambda}{\mu} - \tfrac{1}{2}\,(\varphi_1)_{z=0} = (\nu/\kappa)\dot{\xi}_o\,.$$

On performing the integration with respect to ρ and writing

$$M_1 - iM_2 = \int_o^\infty J_1^2(\lambda a)\,\frac{d\lambda}{\lambda\mu} \tag{25}$$

we finally obtain

$$\dot{\xi}_o = \frac{2A_o - (\varphi_1)_{z=0}}{2\,(\nu/\kappa + M_1 - iM_2)} = \frac{2A_o - (\varphi_1)_{z=0}}{\Psi} = -\left(\frac{\partial\varphi_1}{\partial z}\right)_{z=0} \tag{26}$$

where

$$\Psi = 2\,(\nu/\kappa + M_1 - iM_2) \tag{27}$$

The integrals M_1 and M_2 have been evaluated by King (6), who finds

$$M_1 = H_1\,(2\kappa a)/(2\kappa^2 a)\;;\; M_2 = \{1 - J_1\,(2\kappa a)/(\kappa a)\}\;/2\kappa\,.$$

In the notation introduced by McLachlan (9),

$$G_1 = 1 - J_1(2\kappa a)/(\kappa a)\;;\; G_2 = H_1(2\kappa a)/(\kappa a)$$

so that

$$M_1 = G_2/(2\kappa)\;;\; M_2 = G_1/2\kappa\,.$$

The functions G_1 and G_2 are plotted on page 54 of McLachlan's treatise.

It is seen that the solutions (Equation (2)) derived for infinite partitions satisfy the boundary conditions in the interspaces, so that we can write for the velocity potential φ_n in the nth interspace

$$\varphi_n = A_n e^{-i\kappa(z-nb)} + B_n e^{i\kappa(z-nb)},\; (n = 1, 2, \ldots, \overline{p-1})\,. \tag{28}$$

It is noticed further that Equations (8), (9), (10), (11), and (12) also apply.

The velocity potential for the wave generated by the last panel may be represented by

$$\varphi_p = \int_0^\infty e^{-\mu z} J_0(\lambda\rho) \frac{\lambda}{\mu} f_p(\lambda) d\lambda \, , \tag{29}$$

the origin being at the centre of the last panel and $f_p(\lambda)$ being such that

$$-\left(\frac{\partial\varphi_p}{\partial z}\right)_{z=0} = \begin{cases} \dot{\xi}_p, & 0 < \rho < a \\ 0, & a < \rho < \infty . \end{cases}$$

As before we obtain from this boundary condition

$$\varphi_p = a\dot{\xi}_p \int_0^\infty e^{-\mu z} J_0(\lambda\rho) \; J_1(\lambda a) \; \frac{d\lambda}{\mu} \, , \tag{30}$$

and the dynamical equation for the last partition gives

$$\Psi\dot{\xi}_p = - \Psi \, (\partial\varphi_{p-1}/\partial z)_{z=\overline{p-1}b} = (\varphi_{p-1})_{z=\overline{p-1}b} \, . \tag{31}$$

From Equation (9) it follows that

$$\{(1 - i\nu) \, A \cosh \epsilon_1 - \gamma A \cosh \overline{\alpha + \epsilon_1} + i\nu \, B \cosh \epsilon_2 \} \sinh n\alpha$$
$$+ \; \{(1 - i\nu) \, A \; \sinh \epsilon_1 - \gamma A \; \sinh \overline{\alpha + \epsilon_1} + i\nu \, B \; \sinh \epsilon_2 \; \} \cosh n\alpha \; = \; 0,$$

and the requirement that this must hold for all values of n leads to

$$B = A\gamma \; ; \; \epsilon_2 = \epsilon_1 - \beta , \tag{32}$$

where β is defined by Equation (17).

Returning to Equation (31) and writing

$$\zeta = \overline{p - 1} \; \alpha + \epsilon_1, \tag{33}$$

it is found that

$$\tanh \zeta = \frac{\sinh \beta}{\cosh \beta + (1 - i\kappa\Psi) \; (1 + i\kappa\Psi)^{-1} \; \gamma^{-1}} \, , \tag{34}$$

so that

$$\frac{1 - i\kappa\Psi}{1 + i\kappa\Psi} \, \gamma^{-1} = \frac{\sinh \, (\beta - \zeta)}{\sinh \zeta} \, . \tag{35}$$

On making use of Equations (34) and (35), we may write Equation (26) in the form

$$\frac{A_0}{A} = \frac{(1 + i\kappa\Psi) \; \gamma \; \sinh \beta \; \sinh \, (2 \; \zeta - \beta - \overline{p - 2} \; \alpha)}{2 \; \sinh \zeta} \, , \tag{36}$$

and it follows from Equation (6) that

$$\begin{aligned} \dot{\xi}_p &= (\dot{\xi}_{p-1})_{z=\overline{p-1}b} = - \, (\partial \, \varphi_{p-1}/\partial z)_{z=\overline{p-1}b} \\ &= i\kappa \; (A_{p-1} - B_{p-1}) = 2i\kappa A (1 + i\kappa\Psi)^{-1} \; \sinh \zeta \, . \end{aligned} \tag{37}$$

As shown by King, the radiation output is given by

$$[dW/dt] = \pi a^2 \rho_0 \omega | \, \dot{\xi}_p \, |^2 M_2, \tag{38}$$

and the rate of transmission of energy in the incident wave across the area πa^2 is

$$[dW_0/dt] = \pi a^2 \tfrac{1}{2} \rho_0 | \, A_0 \, |^2 \kappa\omega \, . \tag{39}$$

Hence a "measure of reduction" for transmission through the series of panels is given by

$$\frac{[dW_0/dt]}{[dW/dt\,]} = \frac{\kappa}{2M_2} \left| \frac{A_0}{\dot{\xi}_p} \right|^2 = \frac{1}{32 \; M_2\kappa} \left| \frac{(1 + i\kappa\Psi)^2 \sinh \beta \sinh \, (2\zeta - \beta - \overline{p - 2} \; \alpha)}{\sinh^2\zeta} \right|^2 . \tag{40}$$

As before Kennelly's charts aid in carrying out numerical calculation.

Summary and Conclusion

1. In a semi-infinite sequence of equally spaced, infinitely extended partitions having the same mass per unit area, there are alternate ranges of the frequency corresponding to complete transmission and complete attenuation. The upper frequency ends of the ranges of attenuation are determined by spacing alone, *viz.*, by the equation:

$$\text{Integral number of wave-lengths} = 2 \times \text{spacing};$$

but the widths of the regions decrease with decreasing mass per unit area of the partitions. The regions of attenuation are regions of perfect reflection.

2. In a finite sequence, the transmission is alternately greater and less than that of a single partition of the same total weight, the alternate regions of frequency corresponding to those of the limiting case of a semi-infinite sequence. The effects of viscosity and mechanical damping would probably bring the curve nearer to that for the single partition.

3. The equations obtained for circular panels indicate a behavior somewhat similar to that for infinite partitions although no numerical calculations were made in this case.

Acknowledgment

The author wishes to acknowledge his indebtedness to Prof. L. V. King who suggested this investigation and who has been of great assistance throughout its course.

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REVIEWS AND NOTES

Note on the β- and γ-Rays of Radium D

It has been found in this laboratory that of every 100 atoms of Radium D disintegrating, approximately 3.5 emit a primary γ-ray and 25, L radiation, presumably characteristic of atomic number 83. Hevesy and Lay (1) have shown that for this atomic number, the fluorescent yield of L radiation is about 40% and of M radiation 24%. If it is assumed that this is true for the internal absorption of γ-rays, it is found that the total number of β-rays ejected from L levels is 25/0.40, or 62%. As the number of β-rays ejected from the M and N levels is approximately 60% of that from L levels, the number of β-rays ejected from M and N levels will be 0.60×62, or 37%. As $62+37+3.5=102.5$, there is strong evidence that every atom of Radium D on disintegrating emits a γ-ray, a point of some importance. The internal conversion coefficient of the γ-rays is 0.62 for the L levels and 0.37 for the M and N levels, coefficients that are much higher than those usually accepted. The number of tertiary β-rays will be $62-25$, or 37, with energies corresponding to differences between the L and M and L and N levels, at least 0.76×62 and 0.76×37, or 75, with energies corresponding to differences between M and N and lower levels. Some of these tertiary rays will have such small energies that they will be very difficult to detect. However, the total number of β-rays emitted by Radium D in 100 disintegrations should be 100 primary, 96 secondary and of the order of 100 tertiary. It is not easy to find experimentally the number of atoms emitting M characteristic radiation as these are so soft. The writer has observed the presence of this type of radiation but has not been able to form a reliable estimate of the number of atoms emitting it.

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Note on the Scattering of X-rays at Small Angles

Three methods have been used in this laboratory for investigating the scattering of X-rays at small angles, less than 5° of arc. In the first, the Soller slit method, the primary beam is defined by a set of parallel slits formed by thin metal sheets and analyzed by a similar set of slits which are attached to a graduated circle, so that the intensity distribution of the scattered radiation may be determined. In the second, the primary beam is defined by two very narrow slits and analyzed by a third slit which can be moved perpendicularly to the direction of the beam. By this method it can be ascertained in the cases of intense scattering whether the beam is broadened or whether there is a scattering of the rays out of the beam. This is called the movable slit method. In the third method, the same three slits are used, but are first put in line and then the third is widened. As this is done, more and more

scattered radiation enters the ionization chamber placed behind the third slit. This is called the absorption method. The second and third methods are suitable only when the scattering at small angles is very intense indeed.

The Soller slit method was introduced here by H. M. Cave, and used by Gray and Cave (2) to show that at 2° of arc the intensity of the scattered radiation from gas molecules was proportional to the square of the number of electrons in the molecule. It was also used by W. H. Zinn in an examination of the radiation scattered by water and aqueous solutions. He showed that with the more dilute solutions the solute behaved as a gas, the effective molecule being approximately the solute molecule minus the displaced water. Miss M. F. McDonald also observed the very important result that artificial graphite scattered X-rays intensely at small angles (it was expected that the scattering would diminish to zero as zero angle was approached). Gray and Zinn (3) continued this work and showed that charcoals in particular displayed this effect. Silicon powder obtained by forming a spark under water between silicon electrodes also showed it.

It appeared that this scattering was somewhat similar to that shown by gases, and later work by W. E. Bennett has shown this to be the case. Using the second and third methods, Bennett has been able to show that there are two types of scattering shown by powders, one similar to that referred to above, and the other shown by microscopic crystalline powders where the scattering is really due to refraction in, and reflection from, the surface of the particles forming the powder. A theory of the refraction in and reflection from, spherical particles has been worked out by von Nardroff (4). He has developed a theory following the method used by Debye (1) to account for the charcoal type of scattering, which explains the facts fairly well. The charcoal is assumed to consist of sub-microscopic particles of diameter of the order 10^{-5} to 10^{-7} cm. The charcoal is then treated as a gas. One of the interesting things that has been found is that the larger particles cannot be entirely solid, for when the charcoal is wetted by gasoline, the scattering from them is practically zero. It is assumed that they consist of chains of smaller particles. The total intensity of the scattered radiation should be proportional to the square of the wave-length of the primary rays, and Bennett has shown this to be true. Some idea of the intensity of this scattering may be obtained from the fact that with a primary wave-length of 2.0 Å, one of the specimens of charcoal used gave a mass scattering coefficient of 30 for angles of scattering between $\frac{1}{2}'$ and 3°, the photoelectric absorption coefficient being 12.0. Artificial graphite, of course, is not a true crystal, but presumably a mixture of graphite crystals and charcoal.

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THE LARVAL DEVELOPMENT OF BRITISH COLUMBIA BRACHYURA

I. XANTHIDAE, PINNOTHERIDAE (IN PART) AND GRAPSIDAE[1]

By Josephine F. L. Hart[2]

Abstract

The larval stages of four species of crabs inhabiting British Columbia are described from series obtained by hatching eggs in the laboratory. In the development of *Lophopanopeus bellus* (Stimpson) there are found to be four zoeal stages, of *Pinnotheres taylori* Rathbun two, and of *Hemigrapsus nudus* (Dana) and *H. oregonensis* (Dana) five. There is only one megalopal stage in each of the species.

Knowledge of the life history of Pacific coast decapod Crustacea is very limited. The free-swimming larvae form an important part of shallow water plankton, and as such are a component of the food of fish and macro-plankton. Although the larvae occur in great numbers near the shore, comparatively little is known of their development, owing perhaps to the fact that it is usually necessary to rear the stages to be at all sure of correct identification, as the larvae are so unlike the adults and in many cases so like one another. They are difficult to rear in artificial conditions, and as far as the writer has been able to determine, complete series of only four species have been recorded to date. In this paper all the larval stages of four more species that have been reared from the egg are described. Various authors have described series, taking the stages from plankton, but this method is obviously less accurate than rearing them from the egg.

Lebour's paper (10) is invaluable for the study of early Brachyuran development. It is a brief monograph of the larval stages of some 35 species of crabs inhabiting the Plymouth area. In America, Hyman (5-7) gives summaries of work done previously by others, and makes original contributions to the knowledge of the larvae of the families Xanthidae, Pinnotheridae and Grapsidae. Aikawa (1, 2) describes some of the zoeal stages of a large number of Japanese crabs, dealing particularly with the determination of characters

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Contribution from the Pacific Biological Station, Nanaimo, B.C., and the Universities of British Columbia and Toronto, with financial assistance in the form of a National Research Council bursary, during 1931-32.
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that might be used for classification. There are a number of other papers describing forms found on both sides of the Atlantic ocean, but the works of these three authors have been of most value in the study of crabs found in British Columbia.

Methods of Rearing

The chief difficulty encountered in rearing decapod larvae is the maintenance of a constant supply of suitable living food. Many of the forms eaten in the natural state, when placed under laboratory conditions, soon die and their decomposition products in the water kill the larvae. The life histories of so few forms in this locality have been worked out that there are practically no records of the spawning periods of the invertebrates, and therefore it is difficult, without considerable research, to determine what larvae are available at the different seasons. Lebour (8-10) was successful in rearing three species of crabs in "plunger-jars" on a diet of *Ostrea*, *Teredo*, *Echinus* and *Pomatoceros*. The megalopa and young crabs were fed on small pieces of the mantle of *Mytilus edulis*. The writer has found the larvae of the native oyster, *Ostrea lurida* Carpenter, satisfactory for feeding, as the larvae are retained for some time in the mantle cavity of the adult and therefore can be obtained in quantity, yet are free-swimming when placed in the water. The veliger larvae of Nudibranchs and the trochophore larvae of the Japanese oyster were also used, but did not prove as suitable as those of the native oyster. The megalopa and young crabs ate finely minced muscle of various molluscs.

Berried females were collected and placed in aquarium jars. When the eggs hatch, the larvae swim to the surface of the water and may be removed with a pipette and placed in large beakers of sea water containing food. The water was aerated by stirring with a glass rod. The larvae were examined daily and fresh water and food given if required, and all dead material and sloughed skins removed. Records of pigmentation were kept and specimens of each stage, as well as cast off skins, preserved.

The Developmental Stages

Few records have been obtained of the length of time necessary for the embryonic development of decapods. According to the writer's observations seven weeks is the shortest period during which the eggs were carried attached to the pleopods of the female, and thus protected, kept free from encrustations and debris, and aerated. The length of time seems to be proportional to the size of the adult. The size of the egg mass and the average size of the eggs vary greatly with the different species.

When the egg hatches, the last embryonic stage, the prezoea or protozoea, is set free. This stage may last for some hours, or in exceptional cases, days, but usually is replaced by the first larval stage soon after hatching. The prezoea is enclosed in a thin membraneous cuticle covering the entire body and appendages. In many species this membrane is produced, and delicately plumed, beyond the invaginated tips of the antennules, antennae and telson.

The spines of the carapace, which are present in the first zoea, are held flat against the body by the cuticle in the prezoea and the setae and spines are invaginated upon themselves. The prezoea is able to swim by jerky movements of the abdomen, similar to the movements of gnat pupae. In all the Grapsoid crabs that have been observed to hatch from the egg, the prezoea lacked the prolongations of the membrane found in other forms.

When the embryonic cuticle is cast off, the first zoea swims to the surface of the water by the powerful beating of the exopodites of the first two pairs of maxillipedes. The zoea has a well developed carapace, which may be produced into a rostral, a dorsal and paired lateral spines. There usually is a pair of short setae on the dorsal part of the carapace, above the heart. The abdomen of the first stage is composed of five segments and the telson. The sixth segment may become separate from the telson in a later stage. The thoracic appendages consist of a pair of large compound eyes, tubular antennules, antennae, bilobed mandibles, two pairs of lamellar maxillae and the first two pairs of maxillipedes. The exopodites of the maxillipedes are equipped in the first zoea with four two-jointed, long, plumose setae. The remainder of the thoracic appendages appear as small knobs posterior to the maxillipedes and are covered by the carapace. There are no functioning appendages on the abdomen, but paired swellings may be present on the second to the fifth segments—the pleopods of the post-larva. There is always a lateral knob on either side of the second abdominal segment, and there may be similar, usually smaller ones, on the third, fourth or fifth segments. There may be spines on the lateral posterior angles of the second to fifth segments. The telson is typically bicornuate, with three pairs of internal setae and varying numbers of external spines. The number of internal setae often increases in the later stages and the spines may decrease in number.

When the first zoea moults and the second emerges, the number of setae present on the appendages is found to have increased in most cases. Six is the usual number of setae on the exopodites of the swimming maxillipedes in the second zoea, eight in the third, ten in the fourth and twelve in the fifth. In those forms having only two zoeal stages the non-functioning appendages are of the same degree of development as those of the last zoea of the forms with four or five stages.

The last zoea metamorphoses into the post-larva; the megalopa. It resembles the adult crab in shape and the appendages are all functional. The megalopa can swim freely through the water by means of well developed pleopods, bearing long plumose setae. The abdomen may be flexed under the thorax when the pleopods are not in use. There may be large spines on the carapace and hook-like knobs on the coxae of some of the periopods. In all those crabs that have been reared from the egg only one megalopal stage has been found, the first young crab stage appearing after the first moult of the megalopa. The first young crab stage is like the adult in structure, but usually of different shape and proportions. By subsequent moults the specific characters of the adult are gradually assumed.

Decapod larvae are usually very transparent when alive. The amount and color of the pigment varies according to the degree of contraction of the chromatophores. In some forms the chitin itself may be tinged with bright colors. As the color does not preserve well, it is only of value in preliminary sorting of living or recently killed material, especially in those forms that are morphologically similar but differ in coloration.

The length of time spent in each stage seems to depend considerably on the relative abundance of food, the temperature and salinity of the water and other such external conditions. The first zoeal stage, in suitable natural conditions probably lasts for two or three days, and the time spent in each stage increases as the larva grows. Under laboratory conditions, four to five weeks is usually required for development from the egg to the young crab stage.

The larvae vary considerably in size, so that measurements are only relative. That this variation is not entirely due to the food supply is shown by the lack of uniformity in size and color of the brood hatched by one female.

In attempting to find characteristics suitable for classification, it is necessary to use those that do not become greatly modified as the animal develops. The spines of the carapace, the armature of the abdomen and telson, and the form of the antennae are perhaps the most important. Aikawa (2) uses the number of setae on the endopodites of both maxillae and of the second pair of maxillipedes. The form of the rostrum, the spines on the carapace, the presence of sensory setae on the last pair of legs, and the number of setae on the exopodites of the uropods are characteristic of the megalopae.

Family Xanthidae

The larval development of a number of species of the family Xanthidae has been followed by other workers. Hyman (7) describes some of the stages of forms found at Beaufort, North Carolina, and compares them with European species described by other authors. Lebour (10) gives the characters of the larvae of the family and the description of stages of three species. Connolly (4) gives an account of the larval stages of *Rhithropanopeus harrisi* (Gould) from New Brunswick. On the Pacific, Aikawa (1) studied the first zoeae of two species of *Xantho* found in Japan.

The zoeae and megalopa of *Lophopanopeus bellus* (Stimpson) described below, fit into Lebour's (10) characterization of the larvae of the family Xanthidae, sub-family Xanthinae, with the exception of the number of lateral spines on the telson of the zoeae. The telson resembles that of *Rhithropanopeus harrisi* (Gould), in having only one lateral spine on each fork.

Lophopanopeus bellus (Stimpson)

Eggs carried in April; hatching May to August, changing in color from deep purple to light brown, and increasing in size from 0.33 to 0.41 mm. in diameter.

Typical prezoea, four zoeae and one megalopa. First young crab stage obtained in laboratory five weeks after eggs hatched. Second died five weeks later when attempting to cast its skin.

Prezoea similar in color to first zoea. Spines of carapace flattened against body. Two embryonic spines, of unequal length, enclose antennule. Exopodite of antenna covered by four hairy projections of cuticle. Telson with seven embryonic spines on each side, all but fourth plumose.

First zoea (Fig. 1, *A*) 1.5 mm. (measured from tip of the telson fork to front of head) and 1.5 mm. from tip of dorsal spine to end of rostral. Body tinged with yellow, and rostral spine, antennae and telson forks with russet. Black chromatophores on bases of antennules, on mandibles and maxillae, at bases of lateral and dorsal spines, on postero-lateral part of carapace, on distal part of bases of first and second maxillipedes, and around proximal part of intestine. Pair of chromatophores present at junction of all abdominal segments. A red pigment spot on dorsal spine, a pair on first abdominal segment and on telson.

Dorsal and rostral spines long and tapering, laterals short. No setae on margin of carapace. Abdomen (Fig. 1, *B*) composed of five segments and telson, with knobs on third as well as second segment, and sharp points on postero-lateral margins of third, fourth and fifth. Dorsal part of each fork of telson with one spine and the usual six setae on internal margin.

Two long aesthetes and one seta on tip of conical antennule (Fig. 1, *C*). Antenna (Fig. 1, *D*) characteristic of family: protopodite swollen and produced into a long tapering process, sub-equal to or slightly longer than rostral spine; exopodite minute, about 0.025 mm. long with a short hair at tip. Mandible (Fig. 1, *E*) bilobed; incisor cut into two teeth and molar with a broad cutting surface. Maxillule (Fig. 1, *F*) rather narrow; endites of protopodite with 8 and 4 bristles, segments of endopodite with 1 and 6. Maxilla (Fig. 1, *G*) has 7 and 9 setae respectively on coxopodite and basipodite, 8 on endopodite and 5 soft hairs on scaphognathite.

First maxillipede (Fig. 1, H) typical, with 8 bristles on basis, 4 natatory hairs on exopodite and 2, 2, 1, 2 and 5 on the five joints of endopodite. Second *maxillipede (Fig. 1, I) with 4 bristles on basis, 4 on exopodite and 1, 1 and 5* on endopodite. The remaining thoracic appendages are small and there is *no indication of pleopods on the abdomen.*

Second to Fourth Zoeae

Second zoea (Fig. 1, *J*) about 2 mm. long and 2.1 between tips of spines; third zoea (Fig. 1, *K*) 2.8 and 3.1 mm., and fourth (Fig. 1, *L*) 3.0 and 3.5 mm. As the zoea develops the dark chromatophores become more distinct and branched. Color of dorsal spine and antennae becomes concentrated in a russet band almost at tip of spines. With live material these brightly colored bands on the transparent spines serve as a distinctive character, which is unfortunately lost on preservation.

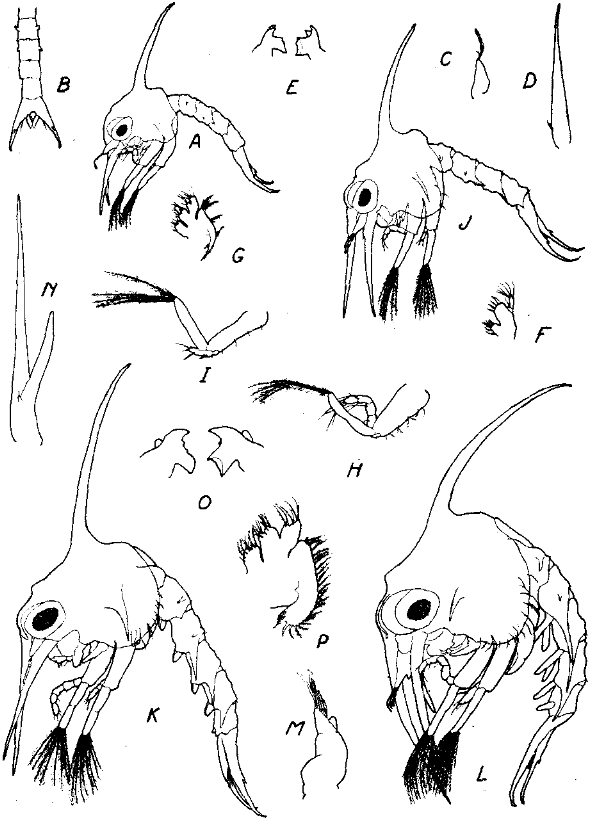

FIG. 1. *Lophopanopeus bellus* (Stimpson). *A, first zoea* × *30; B, abdomen* × *30; C, antennule* × *60; D, antenna* × *60; E, mandibles* × *60; F, maxillule* × *60; G, maxilla* × *60; H, first maxillipede* × *60; I, second maxillipede* × *60; J, second zoea* × *30; K, third zoea* × *30; L, fourth zoea* × *30; M, antennule* × *60; N, antenna* × *60; O, mandibles* × *60; P, maxilla* × *60.*

As the zoea grows the spines on the carapace and abdomen become much elongated. The base of rostral spine is produced laterally to form a shelf curved over bases of eyestalks and there is a distinct median ridge dorsal to this on the carapace. Rostral spine and processes of antennae are subequal in second zoea but in later stages rostral spine over-reaches antennae. Dorsal spine more than doubles in length during development but lateral spines become less conspicuous as zoea grows. Plumose hairs on margin of carapace increase from two in second zoea to about 12 in last. Sixth abdominal segment becomes separated from telson in third stage, although indication of the segmentation may be seen in second. Lateral marginal teeth on third, fourth and fifth abdominal segments gradually increase in size until they become longer than the segments against which they lie. An additional pair of small setae appear on internal margin of telson in third zoea.

Aesthetes of antennule increase in number during development; endopodite in fourth zoea (Fig. 1, *M*) can be seen as small knob, and basal part of appendage becomes inflated. The anlage of flagellum of antenna first seen in third stage; in last zoea (Fig. 1, *N*) it is less than half length of protopodite process. A small, unarmed palp present on mandible (Fig. 1, *O*) of last zoeal stage. Setae on inner margin of protopodite of maxillule increased in number, with a densely plumose seta appearing on outer margin in second stage and a bristle in addition in third; endopodite with 1 and 6 setae in all stages. Maxilla (Fig. 1, *P*) large and lamelliform, with increasing numbers of setae on all parts except endopodite, which bears 8 setae throughout. Swimming setae of maxillipedes increase to 6 and 7 in second zoea, 8 and 9 in third, and 9 and 11 in fourth. The slight division into two parts of third maxillipede and chela which can be seen in second zoea, becomes quite distinct in third. All thoracic appendages well developed in fourth zoea although not functional.

MEGALOPA. (FIG. 2, *A*)

Length about 2.8 mm., carapace 1.5 by 1.3 mm. Yellowish in color with brown pigment in liver and around mouth parts; small patches of russet on legs, mainly on carpi and meri, and on fourth and fifth abdominal segments; branching black chromatophores on eyestalks, on mouth parts, on rostrum, around heart, on postero-lateral part of carapace and between segments of abdomen except first and second, and sixth and telson.

Covered with hairs which are especially dense on distal parts of periopods. Front wide, with a broad tooth near junction of front and sides of carapace; margined with coarse setae. Rostrum blunt, pointing downwards. Surface of carapace depressed in median anterior part; bearing rounded prominences dorsally. Eyestalks elongated. Sixth abdominal segment smaller than others; telson (Fig. 2, *B*) wider than long and usually bearing three plumose setae on posterior margin.

Antennule (Fig. 2, *C*) now composed of peduncle and two flagella: first segment of peduncle swollen to lodge statocyst; third with three long terminal

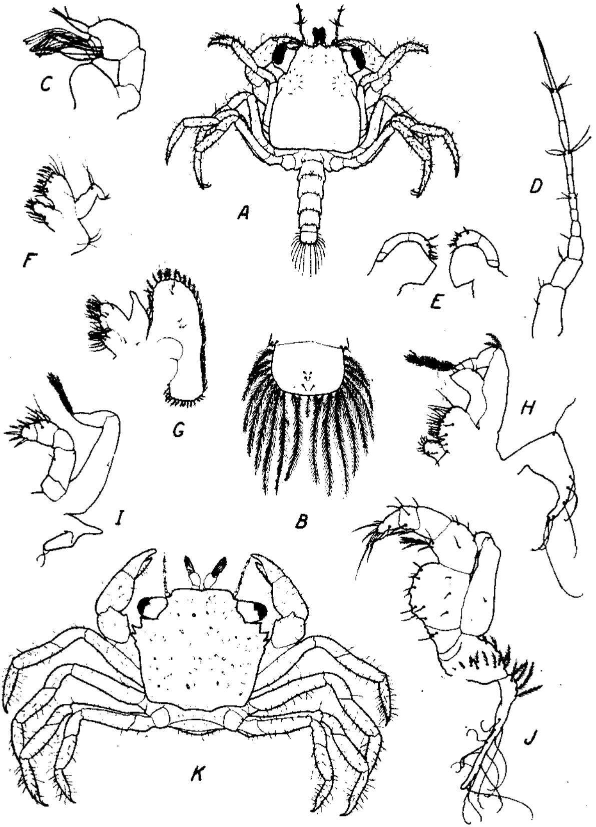

FIG. 2. *Lophopanopeus bellus* (Stimpson). *A, megalopa* × *15; B, telson* × *60; C, antennule* × *60; D, antenna* × *60; E, mandibles* × *60; F, maxillule* × *60; G, maxilla* × *60; ', first maxillipede* × *60; I, second maxillipede* × *60; J, third maxillipede* × *60; K, first ung crab stage* × *15.*

setae, an unjointed flagellum bearing four setae and a segmented flagellum of three broad, short segments bearing aesthetes, and one longer, narrow, terminal segment with one bristle at tip. Antenna (Fig. 2, *D*) of about 11 segments: the proximal three enlarged to form a peduncle; and long bristles on distal segment, penultimate and fourth from tip. Mandible (Fig. 2, *E*) with two-jointed palp, bearing eight bristles on second joint: cutting surface of left mandible smoothly rounded, right coming to a point medially. Endopodite of maxillule (Fig. 2, *F*) decreased in size: now composed of a single segment with distal third twisted upon itself and bearing a few scattered bristles. Endopodite of maxilla (Fig. 2, *G*) somewhat degenerate, with only six soft plumose hairs on the proximal external margin, exopodite with a few setae on surface of scaphognathite as well as numerous marginal ones.

First maxillipede (Fig. 2, *H*) modified, as no longer a swimming appendage: endites of protopodite lobed and fringed with bristles; segmentation of endopodite lost, but a few small setae present distally; exopodite of two segments, the first with two soft hairs terminally, second with four; epipodite well developed. Second maxillipede (Fig. 2, *I*) has a five-jointed endopodite, with strong spines on terminal segments; exopodite two-jointed with five terminal hairs; small epipodite and a lateral projection that develops into a gill in next stage. Third maxillipede (Fig. 2, *J*) resembles that of adult, with flattened and swollen merus and ischium, margined with wide teeth on inner side; epipodite large, but exopodite not well developed. Chela stout, with large spine, the tip of which is bent medially, on ischium. Dactylus of fifth periopod with three slightly specialized setae, but not comparable to those found in some other families. Endopodites of first four pairs of pleopods have three hooked setae on distal internal margin; endopodites of fifth pair of pleopods (uropods) not developed; exopodites with 16, 16, 15, 13 and 8 (Fig. 2, *B*) long plumose setae.

First Young Crab Stage (Fig. 2, *K*)

Carapace about 1.7 by 2 mm. Similar in color to megalopa, with russet chromatophores on walking legs, giving a striped appearance when observed from above. A black chromatophore above heart, and on sternum at base of each cheliped; on ischium of third maxillipede is a bright orange spot, which is characteristic of the species.

Carapace with a broad frontal region, slightly indented medially, and also on lateral margin above eyes; three large teeth and one small one on lateral margin. Surface of carapace covered with minute hairs and scattered, longer setae.

Antennule like that of megalopa except that internal flagellum now composed of two segments, and outer of several indistinct joints with sensory hairs and three without aesthetes. Antenna similar, but more elongated. Now three segments on palp of mandible, second one bearing two setae and third 9-11. Terminal setae only, on endopodite of maxillule. Basipodite of maxilla elongated and exopodite broader with a number of setae on surface.

Plumose setae on inner margin of endopodite of first maxillipede and number increased on distal margin; epipodite larger. More setae on second maxillipede; gill developed. Merus and ischium of third maxillipede flatter, with sharp teeth on medial and distal margins.

Family Pinnotheridae

Hyman states, in reference to the family Pinnotheridae, that the complete history of metamorphosis has not been followed in any species. In his paper (5), he incorporates the work done on the group by authors previous to 1925 and adds descriptions of the early stages of two *Pinnotheres* and a *Dissodactylus* from the Atlantic coast of North America. He found a lack of uniformity in structure and in the number of zoeal stages. When the zoeae described by Aikawa (2) as "*Dissodactylozoea*" and "*Pinnozoea*" are assigned to their correct parental species, some will probably be the larvae of *Pinnotheres*, although superficially the larvae of *P. taylori* Rathbun resemble "*Grapsizoea brevispinosa*".

Lebour (9) describes the only *Pinnotheres* megalopa that has as yet been identified. This megalopa, *P. veterum* Bosc., differs in several respects from that of *P. taylori* described below.

Pinnotheres taylori Rathbun

A berried female, apparently of this little known species, was obtained from a transparent tunicate, March 16, 1933. Straw-colored, with bright orange eggs visible through the abdominal wall. Eggs in early stage of development and did not hatch until the first week in May; mass changing in color gradually to brown, and in size from 0.35 to 0.42 mm. Larvae when hatched covered with a spineless cuticle, shed with appearance of first zoea.

Two zoeal stages and one megalopal. First adolescent stage emerged four weeks after hatching in the laboratory. Zoeae cream-colored, with dark brown chromatophores at base of dorsal spine, around eyestalks, at base of antennules, on mandibles, on carapace postero-laterally, about the centre of basis of first maxillipedes and at junction of all segments of abdomen.

First zoea (Fig. 3, *A*) about 1.3 mm. long and 1.0 mm. between tips of spines. Rostral and dorsal spines rather short and blunt; laterals missing, but a tooth at postero-lateral margin of carapace, which is large and covers all except tips of swimming maxillipedes. Abdomen (Fig. 3, *B*) composed of five segments and telson; with sub-equal lateral protuberánces on second and third segments; fourth and fifth segments somewhat swollen laterally, sides of telson (Fig. 3, *C*) parallel to one another, and no lateral spines, but surface covered with minute hairs, grouped in threes and fours, concentrated on telson forks, (which are very sharp pointed) so as to make them appear finely spined; six internal setae.

Antennule (Fig. 3, *D*) typical. Antenna (Fig. 3, *E*) small, with a short spinous process of protopodite, a swollen area (endopodite), and no exopodite. Mandibles like *Lophopanopeus bellus* but cutting surface rougher. Maxillule

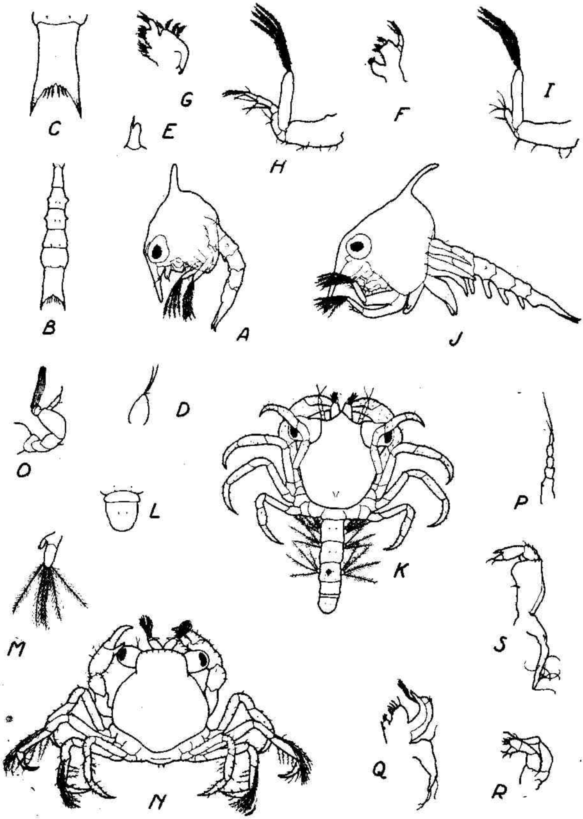

FIG. 3. *Pinnotheres taylori* Rathbun. *A, first zoea* × *30; B, abdomen* × *30; C, telson* × *60; D, antennule* × *60; E, antenna* × *60; F, maxillule* × *60; G, maxilla* × *60; H, first maxillipede* × *60; I, second maxillipede* × *60; J, second zoea* × *30; K, megalopa* × *30; L, telson* × *60; M, fourth pleopod* × *60; N, first young crab stage* × *30; O, antennule* × *60; P, antenna* × *60; Q, first maxillipede* × *60; R, second maxillipede* × *60; S, third maxillipede* × *60.*

(Fig. 3, *F*) usually with five spines on coxopodite and basipodite, and four on distal segment of the two-jointed endopodite. Endites of protopodite of maxilla (Fig. 3, *G*) bear six setae, endopodite three and exopodite four.

Exopodite of first maxillipede (Fig. 3, *H*) with 4 plumose setae; basis with 8 bristles; and endopodite with 1, 2, 1, 2 and 5. Second maxillipede (Fig. 3, *I*) correspondingly with 4; 4; and 0, 5 setae. Rest of thoracic appendages hidden beneath carapace; indication of two parts of third maxillipede (exopodite very small) and chela of first periopod. Anlages of four pairs of pleopods.

SECOND ZOEA (FIG. 3, *J*)

Length about 1.6 mm. and 1.3 between tips of spines. Several setae on lateral part of carapace and two dorsally. Abdomen still of five segments and telson, and no additional internal setae.

Lateral extension of antennule indicative of endopodite of megalopa; aesthetes increased in number and in two rows. Endopodite of antennae enlarged, so longer than protopodite. Mandible with cutting surface divided into numerous small teeth. Plumose hair on external margin of protopodite of maxillule. More setae on maxilla, and three plumose projections on posterior part of scaphognathite.

Exopodite of maxillipedes with six swimming setae. Thoracic appendages becoming fully developed before zoea metamorphoses into megalopa. Pleopods greatly elongated but only four pairs.

MEGALOPA (FIG. 3, *K*)

Differs from *P. veterum* in presence of a small rostrum, a dorsal spine and a rudimentary sixth abdominal segment. Mustard-yellow in color, with transparent periopods. Black chromatophores on median part of carapace just posterior to eyes, and behind dorsal spine; also on each side of dorsal spine and between abdominal segments. On each segment (except dactylus of all but cheliped) of every leg is at least one spot of russet, usually medially placed. Ventrally, a black spot at bases of antennules and antennae, on mandibles, and at junction of chelipeds to sternum; russet at proximal junctions of other periopods. Carapace about 0.7 by 0.5 mm., with a small blunt spine on median posterior region.

Antennule large, with well developed peduncle, with distal segment especially elongated, and flagella rather small, with few setae. Antenna of about six segments; the distal one with one long and one short bristle, and the penultimate, one long seta. Apparently no palps on mandibles. Endites of protopodite of maxillule bear short, stiff spines on inner margin and one seta on external; endopodite rudimentary. Coxopodite of maxilla with six long plumose setae, and basipodite about nine short ones; endopodite quite degenerate, and exopodite with 20 setae around margin.

Setae few on first maxillipedes: four or five on each endite of protopodite, three on shrivelled endopodite, two on exopodite and epipodite. Second maxillipede with poorly developed exopodite, bearing three short setae

terminally; dactylus of endopodite inserted on proximal lateral margin of propodus, instead of terminally. Exopodite of third maxillipede small, with only one distal seta: merus and ischium fused, and dactylus placed in position similar to that of second maxillipede; epipodite small. Chela large and strongly developed. No setae on dactylus of last pair of periopods as is usual in Brachyrhyncha, but which seem to be lacking in all the Pinnotheridae. Only four pairs of pleopods, as none on small sixth abdominal segment (Fig. 3, *L*): endopodite of each pleopod (Fig. 3, *M*) bears two short curved setae, and exopodite, six very long plumose hairs.

First Young Crab Stage (Fig. 3, *N*)

Small, carapace about 0.75 by 0.7 mm. Straw-colored, with periopods a shade deeper in color than body. Front broad, and fringed with about 10 short setae; no trace of dorsal spine of megalopa. Young crab able to swim freely—as does adult—by means of long plumose setae on distal segments of third and fourth pairs of legs.

Antennule (Fig. 3, *O*) large, with distal segment of peduncle swollen and elongated. Antenna (Fig. 3, *P*) of about five segments, with two bristles at tip. Maxillule and maxilla like megalopa. First maxillipede (Fig. 3, *Q*) has a few more setae than megalopa and is quite large. Second maxillipede (Fig. 3, *R*) little changed. Merus-ischium of third maxillipede (Fig. 3, *S*) swollen and flattened distally and dactylus inserted more distally than in megalopa; setae on distal part of epipodite. Chela large and sparsely covered with setae, as are all periopods. Swimming setae present on distal part of carpus and propodus of third and fourth legs.

Family Grapsidae

Hyman (6) found the larvae of the family Grapsidae quite uniform in structure. Aikawa (1), however, observed that the larvae of some of the Japanese forms did not agree with the earlier described types, either in the absence of lateral spines on the carapace, or in the length of the antennae. The development of the two Grapsoid species in British Columbia is similar to that of related forms in Japan. The megalopae that have been described, (with the exception of the first one attributed by Cano to *Pachygrapsus marmoratus* (Fabricius), and which has a large rostrum), are very like those obtained by the writer. There are no sensory setae on the last periopods of the megalopae in Cano's drawings, which is perhaps an oversight, as they are present in most Brachyrhyncha. Rathbun (11) gives figures of megalopae, which are designated as "Grapsoid", *Pachygrapsus crassipes* (?) and *Sesarma magdalensis*, all of which are of the same type as those of *Hemigrapsus* described below.

The first zoea of *Hemigrapsus longitarsis* (Miers), as described by Aikawa (1), is similar to the larvae of *H. nudus* (Dana) and *H. oregonensis* (Dana). It is slightly smaller and there are two extra setae on the endopodite of the first maxillipede. The distribution of the setae differs on the joints of the second maxillipede.

Hemigrapsus nudus (Dana)

Berried females found commonly in April and May at Departure Bay, British Columbia; rarely in June, but reported by Bovard and Osterid (3) to be carrying eggs in June and July at Friday Harbour, Washington. Freshly laid egg mass purplish-black, changing to gray when ready to hatch. Increase in size from 0.38 to 0.45 mm. in diameter.

Five zoeal stages and one megalopal; prezoeal almost disappeared; embryonic cuticle, shed on hatching, has no embryonic spines on antennae or telson, but spines and setae invaginated upon themselves typically.

First zoea (Fig. 4, *A*) about 1.65 mm. in length and 1.2 mm. between tips of spines. Very transparent, with a yellow tinge to body and dark chromatophores: pigment spots around eyestalks, one on carapace between eyes, on labrum, on bases of antennules and antennae, on mandibles, on distal part of bases of first maxillipedes, and at bases of dorsal and lateral spines; some pigmentation at joints of each segment of abdomen and around proximal part of intestine. On dorsal spine and also on fourth abdominal segment, red chromatophores. Rostral spine and forks of telson tinged with violet and lateral spines with russet.

First zoea (Fig. 4, *A*) stout, with all carapacial spines well developed. Postero-lateral margin of carapace bears fine teeth and setae. Abdomen (Fig. 4, *B*) of five segments and telson; large knobs on second abdominal segment and smaller ones on third. External spines lacking on telson, but tips of fork cut internally into fine teeth and a row of minute spines on dorsal surface of each cornua; six internal setae.

Antennule (Fig. 4, *C*) tubular-conical, usually with two long aesthetes and one short seta. Process of protopodite of antenna (Fig. 4, *D*) bears two rows of spines distally, is longer than exopodite, and reaches to about distal third of rostral spine; exopodite with three setae about one-third from tip, which is very minutely spined. Mandibles (Fig. 4, *E*) bilobed, each with a broad denticulated incisor process, and a smaller, more sharply divided molar: left mandible with additional tooth on ventral side. Endite of coxopodite of maxillule (Fig. 4, *F*) usually with five plumose setae, basipodite six, endopodite one and five. Endite of coxopodite of maxilla (Fig. 4, *G*) bilobed with six plumose setae, basipodite with seven; endopodite four and narrow exopodite five, soft, densely plumose setae.

Nine bristles on basis of first maxillipede (Fig. 4, *H*), 4 swimming setae on exopodite, and 2, 2, 1, 2 and 5 setae on joints of endopodite. Second maxillipede (Fig. 4, *I*) with 4 setae on basis, 4 natatory on exopodite and 0, 1 and 6 on endopodite. Rudimentary thoracic appendages present as small lobes under carapace.

Second to Fifth Zoeae

All zoeal stages similar in coloration; chromatophores enlarge and branch, and spines become almost colorless as zoea develops. Second zoea (Fig. 4, *J*) about 1.9 mm. long and 1.6 mm. between tips of spines; third (Fig. 4, *K*)

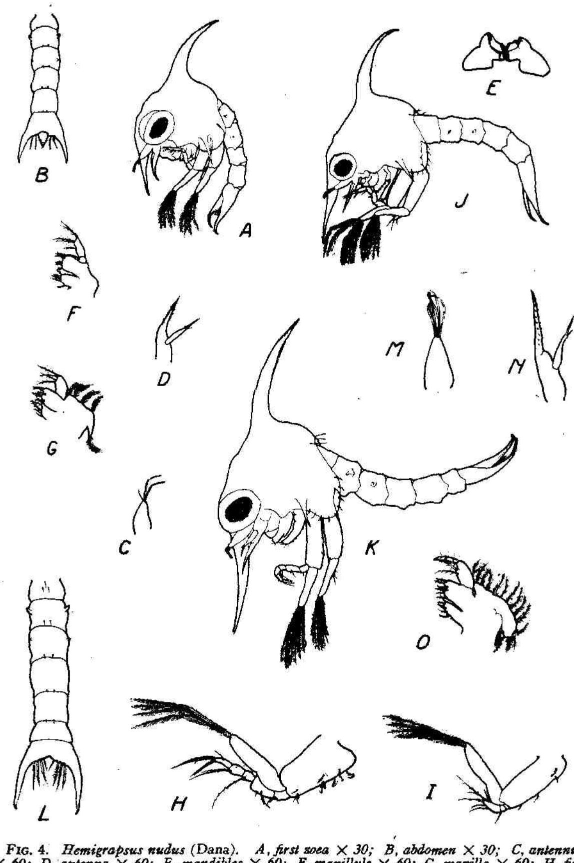

FIG. 4. *Hemigrapsus nudus* (Dana). *A, first zoea* × *30; B, abdomen* × *30; C, antennule* × *60; D, antenna* × *60; E, mandibles* × *60; F, maxillule* × *60; G, maxilla* × *60; H, first maxillipede* × *60; I, second maxillipede* × *60; J, second zoea* × *30; K, third zoea* × *30; L, abdomen* × *30; M, antennule* × *60; N, antenna* × *60; O, maxilla* × *60.*

2.2 and 2.2 mm.; fourth (Fig. 5, *A*) 2.6 and 2.8 mm.; and fifth (Fig. 5, *B*) 3.3 and 3.5 mm. Length of spines as compared to animal as a whole varies with different stages; lateral spines increase very little and in fourth and fifth stages are bent downwards and hardly reach margin of carapace. Marginal hairs increase greatly in size, until in last zoeal stage they are long and plumose; hairs also appear on mid-posterior margin of carapace. Abdomen composed of six segments in second zoea but seventh appears between last segment and telson in third zoea (Fig. 4, *L*); knobs present in all stages on second and third segments; long setae on first segment: one in second stage, three in third, five in fourth and seven in fifth. An additional pair of setae appear internally in telson of third zoea and again in fourth, so that in last two stages five pairs of setae are present. Slight indication of position of pleopods by small swellings on second to fifth abdominal segments of third zoea; in fourth stage increase in size and a small pair appear on sixth segment; in fifth, these anlages long, tubular structures with indications of three parts.

Antennules (Fig. 4, *M*) of second to fourth zoeae similar to first, except that the number of sensory setae increase with age; in fifth zoea (Fig. 5, *C*) indications of adult shape seen: a swelling at base for lodging of statocyst, and a lateral projection, the flagellum (endopodite) distally. Last two zoeae have aesthetes arranged in two series, terminal and sub-terminal. Antennae increase little in size with development, consequently their relative length as compared with rostral spine varies. First indication of flagellum seen in third zoea (Fig. 4, *N*) where a slight swelling occurs between exopodite and spinous process of protopodite; at next moult it reaches nearly to tip of exopodite and finally (Fig. 5, *D*) is sub-equal to protopodite process; indication of segmentation can be seen shortly before metamorphosis to postlarva; spines on tip of exopodite increase in size but never become very clear.

Mandibles of second and third stages like those of first; fourth and fifth have one tooth with a very broad cutting surface; and a small protuberance on fourth zoea mandible enlarges to form the bare, unjointed palp of fifth stage. Setae and spines on endites of maxillule increase slightly in number; appendage becomes stronger but changes little in shape: endopodite (Fig. 5, *E*) of all stages bears one and five setae on its segments; a very densely plumose seta present on external margin of protopodite of all but first zoea and a bare one in addition in the last three zoeae. Fringe of setae on segments of protopodite of maxilla (Figs. 4, *O* and 5, *F*) becomes denser as zoea develops, exopodite enlarges and becomes more plate-like, margined with increasing numbers of densely plumose soft hairs; endopodite with 4 setae throughout.

Swimming setae on tips of exopodites of first maxillipedes are 6 in second stage, 8 in third, 10 in fourth and 12 in fifth; usually 9 setae on basis and 2, 2, 1, 2 and 5 on segments of endopodite, but additional ones found on last three segments of later stages. Setae on exopodites of second maxillipedes correspond in number to those of first; usually 4 setae on basis but these may be reduced to 3 in later zoeae; all have 0, 1 and 6 setae on endopodite. There is an indication of the division into the two rami of the rudimentary third

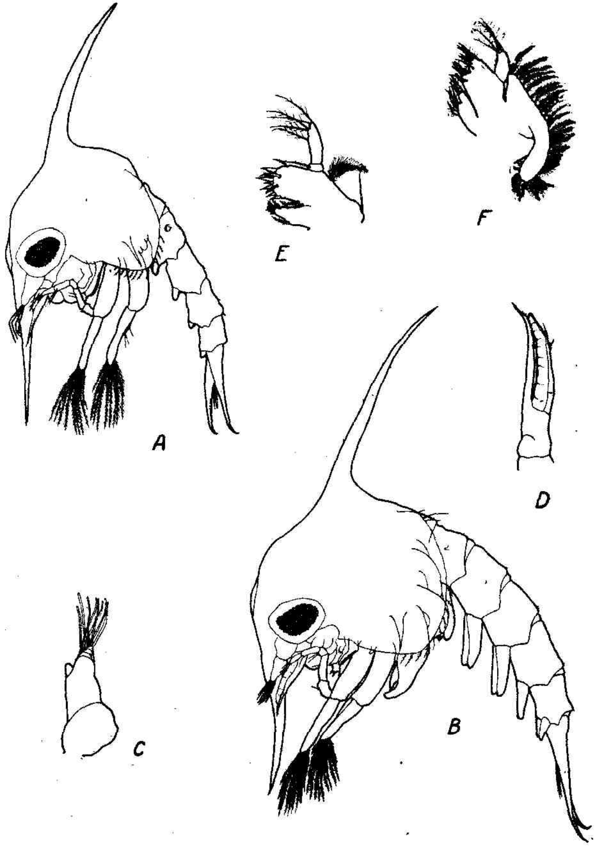

FIG. 5. *Hemigrapsus nudus* (Dana). *A*, *fourth zoea* × *30*; *B*, *fifth zoea* × *30*; *C*, *antennule* × *60*; *D*, *antenna* × *60*; *E*, *maxillule* × *60*; *F*, *maxilla* × *60*.

maxillipede and an indentation at the tip of the first periopod in the third zoea. The segmentation of thoracic appendages can be seen through the enveloping membranes in fifth zoea.

MEGALOPA (FIG. 6, *A*)

Resembles those described and figured by Rathbun (11). About 3.6 mm. in length, and carapace 1.8 to 1.5 mm. Yellowish in color, with scattered black chromatophores: a yellow patch shows through transparent carapace just anterior to gastric mill and another posterior to heart, liver a light brown; dark branching chromatophores on eyestalks, on carapace above gastric region, beneath heart, surrounding intestine, and on joints between all abdominal segments with the exception of the last two; on appendages: on bases of antennae, on mandibles, maxillae, second maxillipedes, coxopodites of chelae and distal part of merus, carpus and propodus and distal part of dactylus of third and fourth legs. Slight amount may be on proximal margin of carpus of fifth leg. Carapace smooth, undulating, with a wide front, the centre of which is depressed where the short, downward-pointing rostrum occurs. Abdomen composed of six segments and telson (Fig. 6, *B*), which is smoothly rounded and bears three or four short plumose setae on distal margin.

Antennule (Fig. 6, *C*) very like that of adult: large swollen base, bearing a peduncle of two segments, the distal one with an unsegmented and a segmented (with sensory hairs) flagellum. Antenna (Fig. 6, *D*) composed of about 11 segments, the distal 3 bearing a number of long bristles. Mandible (Fig. 6, *E*) with strong cutting endite, a two-jointed palp, the second segment of which bears 8-10 short stiff setae. Maxillule (Fig. 6, *F*) like that of adult, with rudimentary endopodite. Endopodite of maxilla (Fig. 6, *G*) now bare of setae and atrophied, but other parts increased in size and well armed with spines and setae.

First maxillipede (Fig. 6, *H*) with well developed endites of protopodite armed with a number of stiff setae; endopodite shrunken and with no definite segmentation; exopodite with four terminal plumose hairs and two at distal margin of first joint; and large epipodite. Second maxillipede (Fig. 6, *I*) has endopodite with five definite segments, terminal ones bearing stiff hairs, and a small epipodite is present. Third maxillipede (Fig. 6, *J*) of typical brachyuran type, with well developed endopodite, exopodite and epipodite. A spur on distal posterior point of propodus of second to fourth legs and three sharp teeth on posterior margin of dactylus. Dactylus of fifth leg (Fig. 6, *K*) bears three long setae, two of which have "combs" in addition to spinules. No hooks on coxae of periopods. Five pairs of pleopods; endopodites of first four pairs each with two stout curved setae and exopodites with 18-20 long plumose hairs; uropods with 9 or 10 long hairs on exopodites.

First Young Crab Stage (Fig. 6, *L*)

Carapace square, about 2 mm. in length and breadth. Front of two curved lobes, unlike the straight frontal margin of adult. Eyes large and extend

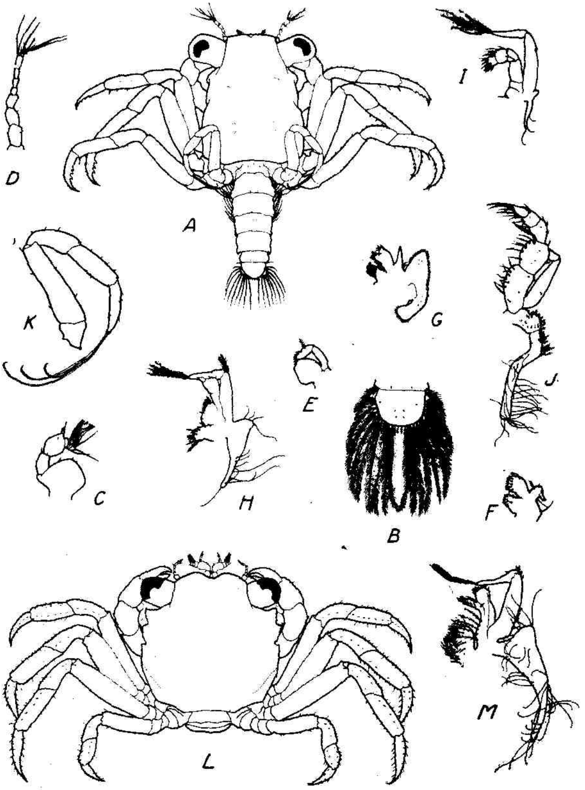

Fig. 6. *Hemigrapsus nudus* (Dana). *A, megalopa* × *15; B, telson* × *30; C, antennule* × *30; D, antenna* × *30; E, mandible* × *30; F, maxillule* × *30; G, maxilla* × *30; H, first maxillipede* × *30; I, second maxillipede* × *30; J, third maxillipede* × *30; K, fifth periopod* × *30; L, first young crab stage* × *15; M, first maxillipede* × *30.*

past sides of carapace, which are parallel for over half their length; three distinct lateral teeth on carapace, the margins cut by minute denticulations. Fine tubercles on carapace and periopods, arranged in definite rows on posterior lateral part of carapace and on chelipeds. All periopods beset with fine hairs, which is unexpected considering the specific character of the mature *nudus*. Usually yellowish in color with varying amounts of black pigment on distal parts of third and fourth legs, and abdomen.

The appendages, with the exception of the first maxillipede and the shrivelled pleopods, are so similar to those of the megalopa that a description of them seems unnecessary. First maxillipede (Fig. 6, *M*), however, shows in endopodite the beginning of the modification to the unique type found in the adult, where endopodite is grooved and tip split so that exopodite lies closely applied and almost surrounded by endopodite.

Hemigrapsus oregonensis (Dana)

Berried females were found near Vancouver, on March 17, 1930, which is some weeks earlier than they have been obtained at Departure Bay, where they hatch from middle of May until August. Early egg mass varies from purplish-black to russet, and when ready to hatch is a light brown. Eggs increase in size from about 0.33 to 0.40 mm.

Zoeal stages five, and megalopal one. First young crab stage has been twice reared, taking four and five weeks. Fourth young crab stage appeared after four and one-half weeks.

The stages correspond closely to those of *H. nudus*. The main distinguishing characters are the smaller size, and absence of a knob on third abdominal segment (Fig. 7, *A*) of the zoeae, and of setae on the posterior margin of the telson (Fig. 7, *B*) in the megalopa. Color seems to be identical in the two species. Dorsal and rostral spines, and abdomen of *H. oregonensis* more slender than *H. nudus*.

Length of different stages (Fig. 7, *C-G*) and distance between tips of spines as follows:

First zoea	1.3 mm. and 1.1 mm.
Second zoea	1.6 mm. and 1.6 mm.
Third zoea	2.0 mm. and 2.0 mm.
Fourth zoea	2.4 mm. and 2.5 mm.
Fifth zoea	2.7 mm. and 2.5 mm.

The development of the zoea is so like that of *H. nudus* that a detailed description seems superfluous. Appendages differ only in size.

Megalopa (Fig. 7, *H*) about four-fifths the size of *H. nudus*, and carapace somewhat narrower in proportion. Length about 2.9 mm. and carapace 1.5 by 1.1 mm. No plumose setae on smoothly rounded posterior margin of telson. Appendages naturally somewhat smaller but in form resemble *H. nudus*. First maxillipede differs slightly; endopodite smaller in proportion, and endites of protopodite bear longer setae on a more rounded margin.

First four pairs of pleopods have 14-17 long plumose setae on exopodite; uropods usually have a soft plumose seta on protopodite and eight or nine on exopodite.

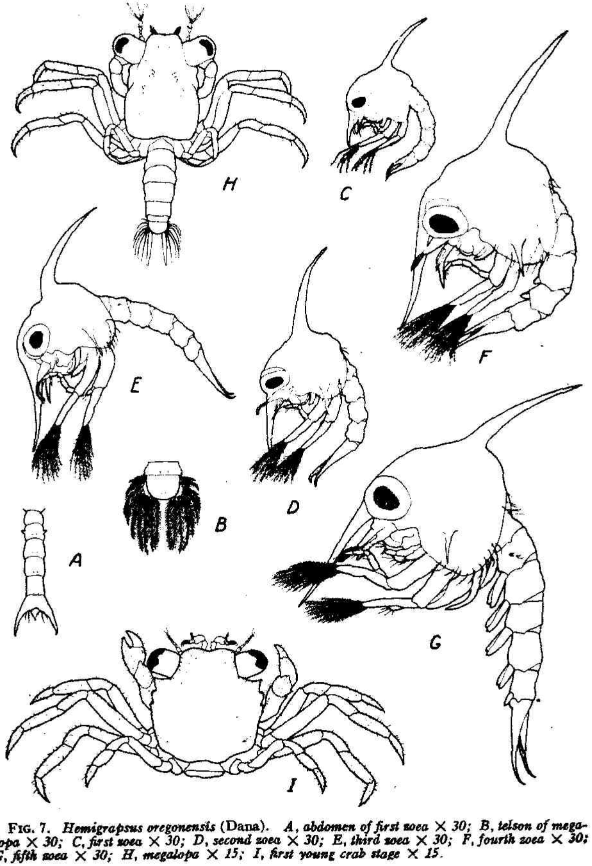

FIG. 7. *Hemigrapsus oregonensis* (Dana). *A, abdomen of first zoea* × *30; B, telson of megalopa* × *30; C, first zoea* × *30; D, second zoea* × *30; E, third zoea* × *30; F, fourth zoea* × *30; G, fifth zoea* × *30; H, megalopa* × *15; I, first young crab stage* × *15.*

First young crab stage (Fig. 7, *I*) also closely resembles that of *H. nudus*. Carapace 1.6 by 1.6 mm. Chelipeds and walking legs not quite as stout as those of the related species.

Acknowledgments

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POST EMBRYOLOGICAL DEVELOPMENT OF EPHEMEROPTERA (MAYFLIES). EXTERNAL CHARACTERS ONLY[1]

By F. P. Ide[2]

Abstract

The development of external structures is followed from hatching to maturity. The life histories of two species *Stenonema canadense* Wlk. and *Ephemera simulans* Wlk. are described in detail and less complete accounts given of nine others.

Each moult results in a change in the number of segments in the caudal filaments and on this basis it has been possible to determine the number of instars. In *Stenonema canadense* the number was found to be between 40 and 45, and in *Ephemera simulans* about 30. Segments are added to the caudal filaments of the former at each moult in the following way: one in each of the first two moults, two in each of the next three, three in the next and four at each subsequent nymphal moult. In the change from nymph to subimago distal segments are dropped in *Ephemera* and probably in *Stenonema* also.

Mouth parts are very different in the newly hatched than in the full grown nymph as described for *Stenonema canadense*.

A definite case of a hypermetamorphosis of the tarsus and its claws appears in the life history of *Epeorus humeralis* and *Iron pleuralis*.

Gills are absent in the first instar in all the species studied. In some species they appear simultaneously on all the gill bearing segments at the first moult. In others they appear on segments five and six only at this moult, those of the other segments appearing only after several moults. The internal or secondary ramus of the gills appears much later in the nymphal life. The ultimate shape of the gill is influenced in some cases by the fact that the gills of segments five and six are segmented or unsegmented.

In *S. canadense* the wing pads make their appearance in about the fifteenth from the last instar and the claspers and external genitalia of the male are apparent in about the eighth from the last instar.

At each moult there is some structural change in the nymph adapting it to the environment. The environmental relation is being constantly changed by increase in size of the organism, thus necessitating these adaptations to preserve an equilibrium.

Introduction

There is relatively little known of the younger stages of Ephemerid nymphs and a study of these is important not only to a better knowledge of the insects themselves but of their distribution and ecology.

Lubbock (1864 and 1867) describes very fully the development of *Cloeon dimidiatum* but has missed at least one of the early instars and misinterpreted the way in which segments are added in the caudal filaments. Joly (1872) describes in brief some of the changes taking place in the nymphs of *Ephoron* (*Palingenia*) *virgo*. (See also Joly (1876).) Vayssiere (1882) describes the development of *Ecdyonurus* (*Heptagenia*) *venosus* (*longicauda*) describing nine stages which, however, do not correspond to instars. Lestage (1921) divides the nymphal life into three stages, stade larvulaire, stade larvaire and stade larva-nymphal. Murphy (1922) has reared *Baetis posticatus* Say and finds that there are 26 instars in this species. Gros (1923) in a study of *Ecdyonurus forcipulus* Koll. has described more stages than Vayssiere, basing the separation mainly on the growth of the gills. Wiebe (1926) describes the first three instars of *Hexagenia bilineata* Say and Neave (1932) the first instar of *Hexagenia limbata occulta* Walk.

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In the present paper nymphal stages of 11 species of mayflies commonly found in the upper reaches of trout streams of Ontario are described. The species are:

1. *Ephemera simulans* Walker;
2. *Stenonema canadense* Walker;
3. *Stenonema fuscum* Clemens;
4. *Stenonema tripunctatum* Banks;
5. *Heptagenia pulla* Clem;
6. *Heptagenia hebe* McD.;
7. *Epeorus humeralis* Morgan;
8. *Iron pleuralis* Banks;
9. *Isonychia bicolor* Wlk.;
10. *Ephemerella subvaria* McD.;
11. *Leptophlebia debilis* Wlk.

The life histories of the first two species mentioned were worked out fairly completely, but even in these all the instars were not determined. In both cases, however, the method of addition of segments in the caudal filaments and antennae was determined. It was found that each moult results in a change in the segmentation of the caudal filaments. The number of instars through which the full-grown nymph has passed may be estimated fairly closely by an examination of the caudal filaments and could be definitely determined in this way if there were not a fusion and obliteration of some of the segments basally. In *Ephemera simulans* the number of instars was estimated at about 30 and in *Stenonema canadense* at between 40 and 45.

For other species listed much less complete accounts are given, especially for *Ephemerella subvaria* and *Leptophlebia debilis*. The description is of the eggs and first instar of the former, and the gills of the latter.

The material was collected from the Mad and Pine rivers, tributaries of the Nottawasaga river, in Dufferin and Simcoe counties, Ontario, in connection with an ecological study of mayflies of these streams conducted during the summers of 1930, 1931 and 1932.

All the species herein treated have already been adequately described in the adult and full-grown nymphal state; references to these descriptions are given in the case of each species.

Method

Nymphs were collected periodically in the stream and examined under a compound microscope in the following way. A film of water was spread over a microscope slide and nymphs of one species placed dorsal side up in rows to facilitate the taking of measurements. When thus arranged the nymphs were covered with a cover slip and water was introduced, care being taken to exclude all air bubbles. The temporary mount thus made was placed on a larger piece of plate glass for convenience in handling under the objective of the microscope. Measurements were made using a squared whipple disc in a 10× Spencer ocular. For the later instars in which the mesothoracic wing pads are growing out rapidly relative to the growth of the nymph as a whole, the length of these pads was measured and also the length of the seventh abdominal tergum. The length of the wing pad was then divided by the length of the seventh tergum of the same individual to give a factor x.

By this means the differences in sizes of nymphs at the same instar were compensated for. As the wing pads grow out, not evenly, but by sudden increases in length at each moult, frequency diagrams made by plotting values of x as abscissas and the number of individuals as ordinates, gave a separation of the later stages into groups. Each group, it was shown, did not always represent one instar, since in one case it was found that there was little increase in length of the wing pads, but that in male individuals the instars could be easily separated by the change in size of the claspers which are growing out at the same time as the wing pads.

It was found that the intermediate stages could be roughly grouped by making a count of the segments in the antennae. Other structures which showed metamorphosis at each moult were the gills, and in the case of *Ephemera simulans*, the mandibular tusks. Very minute changes in morphological structure were found to be very constant and to indicate true instars.

The very early stages were never taken in collections in the stream owing to their small size (less than a millimetre). These were secured by capturing female adults on their ovipositing flight and relieving them of their egg masses which were set to incubate in fresh water in glass containers. Usually it was found that the eggs incubated under these conditions and hatched, even in the case of rapid water forms. With the rapid water forms, however, all individuals died either in the first or second instar. *Stenonema canadense*, and *Ephemera simulans* were reared further, up to the eighth or ninth instar.

Samples were taken from the rearing jars at intervals of two or three days and notes made on all the individuals contained in the sample, special attention being given to the changes in segmentation of the caudal filaments and of the antennae, the change in the number of spines on the femora and the appearance and subsequent increase in size of the gills. These structures were very sensitive indicators of change at moulting, especially the caudal filaments which, it will be shown in this paper, register every moult through which the individual passes.

Examination of the early instars was carried out under the compound microscope, using a 4 mm. Spencer objective and a 10× ocular, and examination of the older stages under a 16 mm. Spencer objective and a 10× ocular. The oil immersion was used to examine the mouth parts of the first and fifth instars of *Stenonema canadense*.

All drawings were made on cards ruled in quarter-inch squares and each is drawn to one of three scales of magnification, designated X, Y or Z, depending on whether the 16 mm., 4 mm., or oil immersion objective was used. The scales of magnification are given in Fig. 2,–13, and the magnification of each figure is mentioned in the explanation of the plate concerned. In any one figure drawings of structures having the same number were made from one individual (except in one or two instances), letters designating the separate structures.

Description of Instars

In the description of the instars of *E. simulans* and *Stenonema canadense* some characters have been tabulated to save repetition. These characters are omitted from the general accounts so that the data in the tables should be consulted as supplementary material.

Ephemera simulans Walker

The full grown nymph of *E. simulans* was described by Clemens (1915). This stream form, which may eventually be shown to be specifically different

TABLE I

DIAGNOSTIC CHARACTERS OF EARLY AND LATE INSTARS OF *Ephemera simulans*

Instar	Length in mm.			No. of segments in antennal flagellum (including terminal piece)	Apparent no. of segments in ant.	No. of segments in caudal filament including terminal piece	Gills	Setae on posterior border of femur
	No. of individuals	Average	Lower and upper limits					
1	(25)	0.79	(0.64–0.85)	3		5	Absent	1 (and hairs)
2	(27)	0.82	(0.72–0.91)	4		5.5	On Segs. 2-7	1 (and hairs)
3	(4)	0.94		5	4	6	On Segs. 2-7	2
4	(4)	0.99	(0.87–1.12)	6	5	7	On Segs. 2-7	
5				7	6	9	On Segs. 2-7	5
6				8	7	12	Gill 7, sec. ramus begun	6
7				9	8	16		12
8								
9				11	10			
?10				12	10			13
11				13	11			
12								
13				15	13			26
14- about 23								

	Length of mesothoracic wing pad of male, mm.	Distance between wing pads at base, mm.	Segments in male claspers	Length of male claspers
?Nonult	0.29			visible
Octult				visible
Septult	0.42	0.40	1	0.05 mm.
Sextult	0.42	0.50	1	0.10
Quintult	0.64	0.50	2	0.10
Quartult	0.74	0.50	3	0.22
Tertiult	1.10		3	0.34
Penult	1.36		3	0.53
Last	2.60		3	0.85

The length of the nymphs is measured from the front of the head to the tip of the abdomen excluding the length of the caudal filaments. Where the length is the average of a number of individuals the number measured is placed in brackets and also the upper and lower limits of the lengths. In recording the segments of the antennae the two basal segments are omitted but the terminal segment is included in the count throughout. Similarly in the count of segments in the caudal filaments the terminal segment is included. The arrangement of setae on the femur refers to the series on the posterior border only.

Some segments are fused basally in the antenna and caudal filament and in these cases the true number and the apparent number are listed.

from typical *E. simulans* of lakes, was present in countless numbers in the slower flowing reaches of the Mad river at Singhampton, Ontario, where its nymphs burrow in the gravel and marl of the bottom in the channel of the stream.

Eggs oblong, about .25 × .15 mm. (Fig. 1,–1). Surface of the egg reticulate as shown (Fig. 1,–1). The egg figured is one from which the nymph had hatched. Eggs extruded in two cylindrical masses, one from each oviduct.

First instar (Fig. 1,–2). Average length of 25 individuals .79 mm. with upper and lower limits of .85 and .64 mm. respectively. Head somewhat pentagonal, front margin forming two sides of pentagon; compound eyes rather less prominent than lateral ocelli and directly behind them; antennae composed of two thick basal segments and terminal flagellum in which are two segments differentiated and a terminal undifferentiated portion about as long as the second segment; hairs present subapically in these segments. Yolky enteron visible as an opaque body (stippled in figure); femora with one rather weak distal seta and two proximal hairs. Gills absent; three subequal caudal filaments, each of five segments of which the three basal are well differentiated and the others indistinct; rosette of spines present apically on Segment 1 only; Segments 2 and 3 armed apically with fine hairs.

Second instar, (Fig. 1,–3, 3a and 3b). Basal segment of antennal flagellum shorter and thicker than in first instar. Gills on Segments 2-7 inclusive as projections of the postero-lateral angles. The basal segment of caudal filament thickened, and shortened, the second segment better differentiated; a rosette of rather weak spines apically as on the basal segment; Segments 3 and 4 longer than in previous instar; Segment 5, the terminal piece more than twice as long as Segments 4 and 5 of the previous instar. The writer is inclined to think that this segment represents two undifferentiated segments; if this is so it means that the equivalent of a segment has been added in this instar to those formed in the previous instar.

Third instar, (Fig. 1,–4, 4a, 4b, 4c). The basal segment of antennal flagellum more elongate than in the previous instar, and probably the product of the two basal segments fused, making five segments in all; on the basal segment of the antenna adjacent to the flagellum a pair of very stout curved setae, which are present in this position in all subsequent stages. Femora each with a distal stout seta on posterior border and immediately proximad of it a weaker seta; gills elongate, uniramous; Gill 7 shown in Fig. 1,–4; on the lateral border of the gill in the distal third, a process, the first of the series of filaments which develop along the lateral border.

Caudal filaments (Fig. 1,–4b) have added one segment to the number already formed in the second instar, so that there are now six segments in all; basal segment much shortened and thickened.

Fourth instar, (Fig. 1,–5, 5a, 5b and 5c). Rostrum (Fig. 1,–5) developed as a shelf with anterior margin convexly curved; seventh gill of right side shown in Fig. 1,–5a. It is about twice as long as in the third instar, still

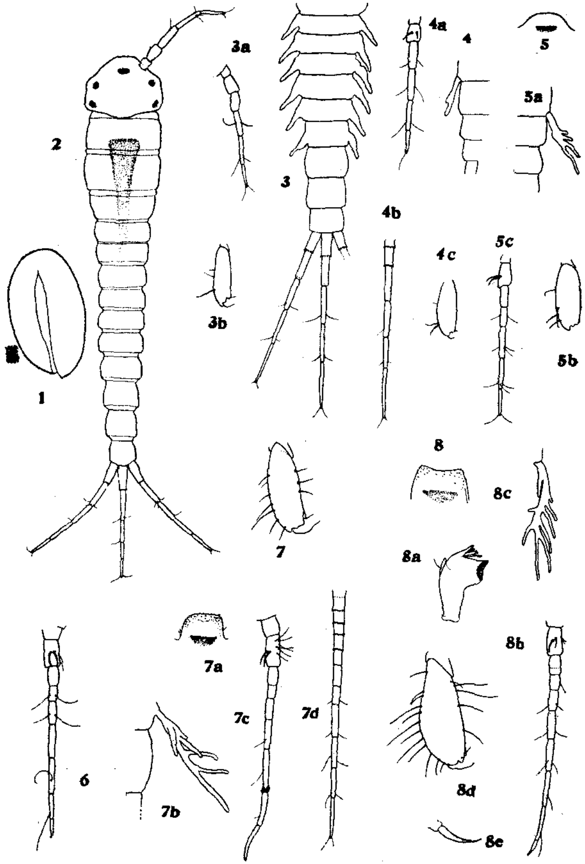

FIG. 1. *Ephemera simulans. All mag. Y. 1, Egg. 2, First instar. 3, Second instar, abdomen; 3a, antenna; 3b, femur. 4, Third instar, Gill 7; 4a, antenna; 4b, caudal filament; 4c, femur. 5, Fourth instar, rostrum; 5a, Gill 7; 5b, femur; 5c, antenna. 6, Antenna, fifth instar. 7, Sixth instar, femur; 7a, rostrum; 7b, seventh gill; 7c, antenna; 7d, lateral caudal filament. 8, Seventh instar, rostrum; 8a, mandible; 8b, antenna; 8c, seventh gill; 8d, hind femur; 8e, tarsal claw.*

uniramous, but with three lateral processes instead of one. The three basal segments of the caudal filaments now armed apically with a rosette of short spines.

Fifth instar (Fig. 1,–6). The rostrum is more truncate than in the fourth instar; caudal filaments with nine segments in flagellum, an addition of two to those present in the fourth instar. Gill 7 with five filamentous appendages but still uniramous.

Sixth instar, (Fig. 1,–7, 7a, 7b, 7c and 7d). At distal end of Segment 5 of the antenna is a thick sclerotized region, and in most individuals of this stage the antenna was broken off at this point. Usually the antennae were broken off at different points, one losing practically the whole flagellum and the other about one third of it. Rostrum (Fig. 1,–7a), more extended and truncate than in previous instar, and its surface roughened with numerous tubercles. Gill 7 more complex and with the beginning of the internal or secondary ramus, (Fig. 1,–7b). Caudal filaments show an addition of three segments in the flagellum, making 12, including the terminal piece.

Seventh instar, (Fig. 1,–8, 8a, 8b, 8c, 8d and 8e). Rostrum slightly emarginate. This condition is forecast in the previous instar figured where the new rostrum is visible through the old nymphal skin. Mandible (Fig. 1,–8a) with tusk well under way in development; tarsal claw long, scimitar-shaped and lacks pectinations.

Ninth instar, (Fig. 2,–9, 9a and 9b). Mandibular tusk shown in Fig. 2,–9; internal or secondary ramus of Gill 7 more elongate and conspicuous than in seventh instar, and the outer or primary ramus more complex.

? Tenth instar, (Fig. 2,–10, 10a, 10b, 10c and 10d). Mandibular tusk more developed, with a rather blunt apex, and a seta basally. Rostrum more concave on its anterior border than in seventh instar. Seventh gill (Fig. 2,–10b) with an elongation and basal enlargement of the internal or secondary ramus.

Eleventh instar, (Fig. 2,–11, 11a, 11b and 11c). Rostrum more emarginate and small setae appearing along anterior border; mandibular tusk longer than in previous instar and acutely instead of bluntly pointed; apically also it is much more heavily sclerotized than in the tenth instar; basally the tusk has two setae, one of which is longer and stronger than the other. Gill of seventh segment shows a change in the secondary ramus in the appearance of a bifid apex and the beginning of one medial filament.

? Thirteenth instar, (Fig. 2,–12, 12a, 12b, 12c and 12d). Rostrum has become more emarginate and six short setae are present around concavity in front of median ocellus. Mandibular tusk with much the same proportions as in previous instar, but longer, more heavily sclerotized and with four long setae proximally Setae on anterior border of femur more numerous than in tenth instar, and a row of seven short, thick setae has appeared in this position. Secondary or medial ramus of Gill 7 increased in length and with seven filaments, an addition of four to those started in the eleventh instar.

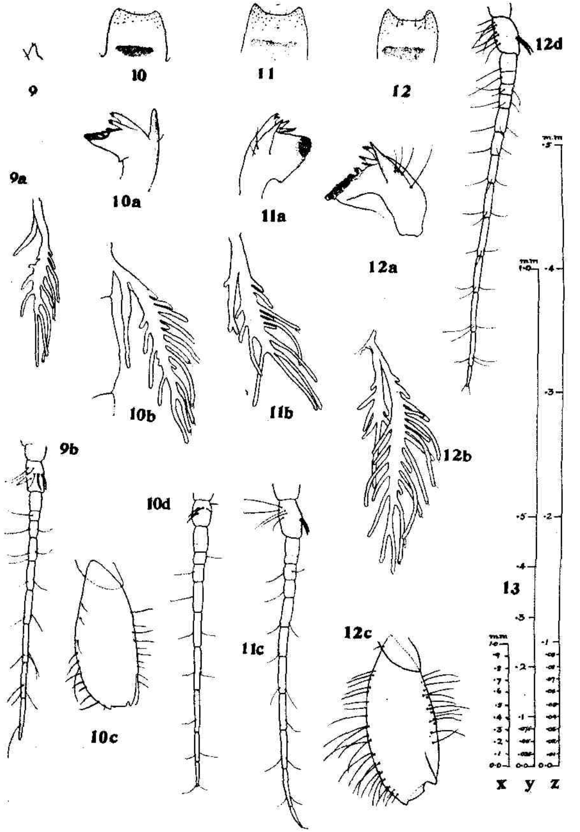

FIG. 2. *Ephemera simulans. 9-12d mag. Y. 9, Ninth instar, mandib. tusk; 9a, seventh gill; 9b, antenna. 10, Rostrum; 10a, mandible; 10b, seventh gill; 10c, hind femur; 10d, antenna. 11, Rostrum; 11a, mandible; 11b, seventh gill; 11c, antenna. 12, Rostrum; 12a, mandible; 12b, seventh gill; 12c, hind femur; 12d, antenna. 13, Scales of drawings with compound microscope using 10X ocular; X, using 16 mm. objective; Y, using 4 mm. objective; Z, using oil immersion*

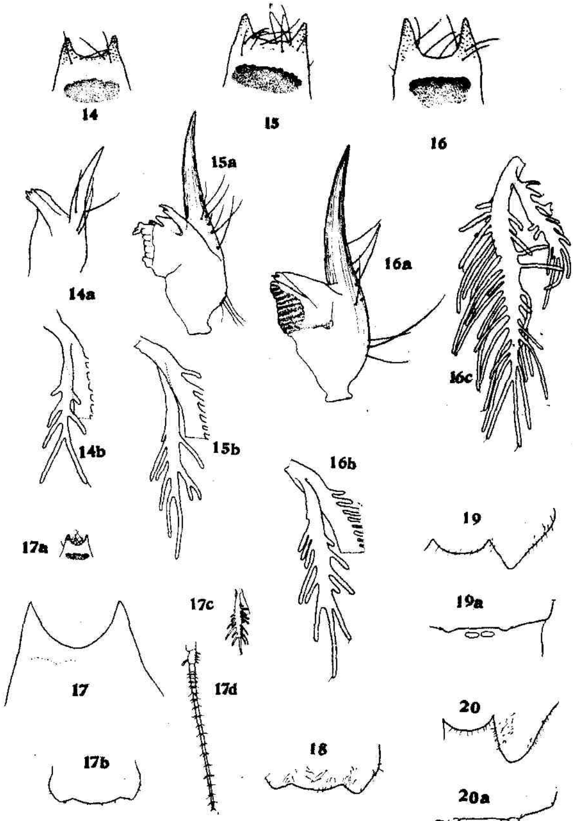

FIG. 3. *Ephemera simulans. 14-17 mag. Y. 17a-20a mag. X. 14, Rostrum; 14a, mandible; 14b, seventh gill. 15, Rostrum; 15a, mandible; 15b, seventh gill (ventral lobe entire); 16, Rostrum; 16a, mandible; 16b, seventh gill; 16c, seventh gill (entire). 17, Rostrum; 17a, rostrum enlarged; 17b, mesothoracic wing pads; 17c, seventh gill; 17d, antenna. 18, Mesothoracic wing pads. 19, Mesothoracic wing pads; 19a, male genitalia. 20, Mesothoracic wing pads; 20a, male genitalia.*

In Fig. 3,–14-16 are illustrated corresponding structures of three later instars which it will be seen at a glance cannot be consecutive instars. Fig. 3,–14, 14a, 14b, are taken from an individual which has 15 segments distinguishable in the antennal flagellum so that it is probably the 17th instar. Fig. 3,–15, 15a, 15b, are of a stage which is probably about the 18th instar, judging by the number of segments in the antennae. Similarly Fig. 3,–16, 16a, 16b, probably represent the 20th instar, since about 18 or 19 segments could be made out in the antennal flagellum. Adding to these one or two lost by fusion at the base the true number is calculated at about 20, and this, as we have seen in previous cases, gives approximately the number of the instar.

Through these stages the rostrum shows a deepening of the anterior concavity until its ultimate bifid character is developed. After the twentieth instar there is little change in its relative shape, merely an increase in size. The tusks of the mandibles also develop rapidly their final proportions during the stages immediately preceding the twentieth instar. The gills of the seventh segment show increasing complexity of structure which is easier to observe in the secondary ramus which alone is shown in full in Fig. 3,–14b, 15b, 16b. In Fig. 3,–16c is shown a complete seventh gill for a comparison of the secondary with the primary ramus. The wing pads have begun to form in this series probably in the stages immediately before the twentieth instar.

In Fig. 3,–17, 17a, 17b, 17c and 17d show structures of a later instar which is probably about the twenty-second, judging by the number of segments in the antennae, the increased size of the rostrum and the greater complexity of the secondary ramus of Gill 7. The wing pads in this instar reach back to a point in line with the posterior extremity of the mesonotum. The figures of this instar and subsequent ones are drawn at a lower magnification (Mag. X Fig. 2,–13), but Fig. 3,–17, of the rostrum is drawn to the same scale (Mag. Y, Fig. 2,–13) as that used in all previous figures as a basis of comparison.

Fig. 3,–18, shows the mesonotum of what is probably the next instar in which the wing pads project back beyond the posterior margin of the notum. There were about 23 segments discernible in the antennal flagellum. Obviously there is a gap between the instar represented in Fig. 3,–18 and that represented in Fig. 3,–19 and 19a.

During the last eight or nine instars the wing pads and male external genitalia are rapidly growing back so that the series is probably more complete, but even so it has some gaps; for instance there is apparently an instar missed between those represented in 19 and 19a, and in 20 and 20a, Fig. 3.

A system of nomenclature used by Dr. P. P. Calvert has been followed for the later instars.

? Nonult instar (Fig. 3,–19, 19a). In this instar the wing pads are triangular and measure about .29 mm. in length. The claspers in the male are present as mere rounded elevations on the posterior margin of the ninth sternum. The penes have made their appearance as elongate, slightly elevated tubercles on the intersegmental membrane behind the ninth sternum.

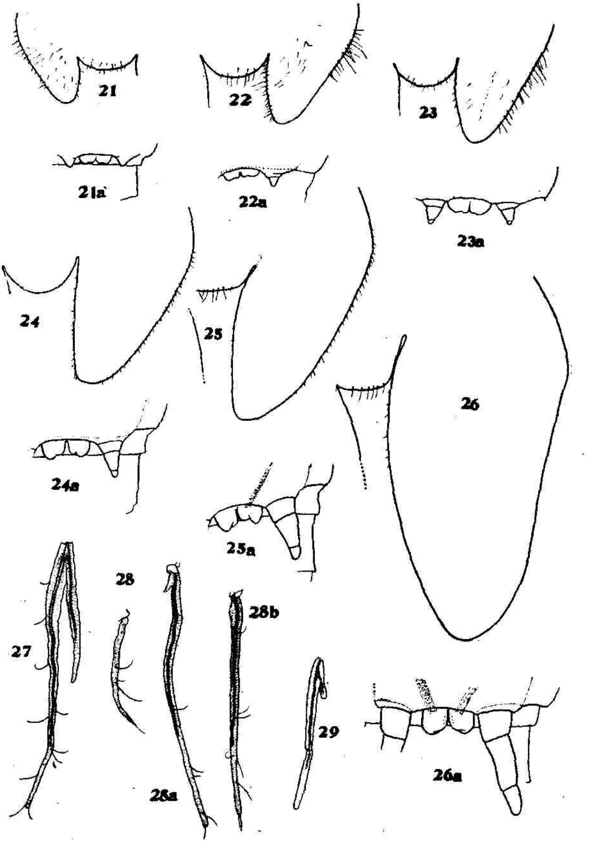

FIG. 4. *Ephemera simulans. 21-26a mag. X. 27-29 mag. Y. 21, Mesothoracic wing pads; 21a, male genitalia. 22, Mesothoracic wing pads; 22a, male genitalia. 23, Mesothoracic wing pads; 23a, male genitalia. 24, Mesothoracic wing pads; 24a, male genitalia. 25, Mesothoracic wing pads; 25a, male genitalia. 26, Mesothoracic wing pads; 26a, male genitalia. 27, Leptophlebia debilis; fifth gill of left side. 26, Leptophlebia debilis first gill; 28a, fourth gill; 28b, fifth or sixth gill. 29, Leptophlebia debilis; sixth gill of left side.*

Septult instar, (Fig. 3,–20, 20a). The penes (penes + parameres) now betray their double nature in the bilobed condition of each.

Sextult instar, (Fig. 4,–21, 21a).

Quintult instar, (Fig. 4,–22, 22a). Wing pads are relatively longer than in previous instar; male claspers about .1 mm. in length, and of two segments, a short basal and a longer conical apical one.

Quartult instar, (Fig. 4,–23, 23a). Wing pads have increased to a length of .74 mm. which is not as great an increase as there was between the two previous instars; distance between wing pads about .5 mm. which is about the same as in the two former instars. The two distal segments of the claspers represent the single apical segment of the previous instar.

Tertiult instar (Fig. 4,–24, 24a).

Penult instar, (Fig. 4,–25, 25a). Wing pads have not increased very greatly in length, measuring now about 1.36 mm. Instead of being directed posteriorly as in previous instar, they converge noticeably at their apices. Male claspers have lengthened and lost some of their conical symmetry, but are still composed of three well defined segments. It is interesting to note that although there has been little increase in the length of the wing pad over that in the last instar, there has been a much greater increase in the length of the claspers.

Last instar, (Fig. 4,–26, 26a). Wing pads increased to a length of 2.6 mm. Towards the end of this instar the wing pads become first grayish and then black, prior to emergence.

The changes taking place in the caudal filaments from nymph to subimago and from subimago to adult are illustrated in Fig. 5.

A count of the single segments in a lateral caudal filament (Fig. 5,–30, 30a) of a female nymph of the last instar gives about 94 segments formed during the thirty or more instars. The subimago (Fig. 5,–32, 32a, 32b) has fewer (about 74) and the adult female (Fig, 5,–33) and male (Fig. 5,–34) have each about 72 segments. In spite of this great reduction in the number of segments the caudal filaments of the adult are much longer than those of the subimago, and those of the subimago are in turn much longer than those of the full-grown nymph. This great increase in length is produced by a great elongation and swelling of the segments, and not by any increase in their number. Indeed, there is actually a reduction in the number of segments in the change from nymphal to subimaginal state. The twelve distal segments of the nymphal caudal filament do not represent segments of the subimago or adult, but are dropped in the manner shown in Fig. 5,–31a, where the developing subimaginal filament could be made out within.

Fig. 5,–31, 31a, of the nymphal filament, show the developing subimaginal filament within and Fig. 5,–32, 32a, show the developing imaginal filament within the subimaginal. In Fig. 5,–30, 30a, the segments formed at each ecdysis are indicated, and in the other figures an attempt has been made to designate homologous segments similarly. A more detailed description of Fig. 5 will be given later in a discussion of segmentation.

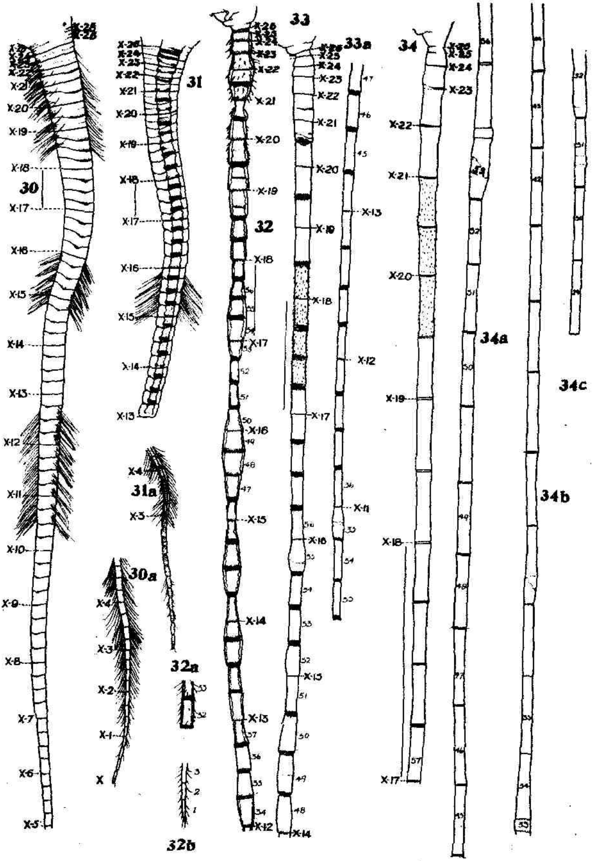

FIG. 5. *Ephemera simulans. All mag. X. 30 and 30a, Lateral caudal filament of female, last instar nymph. 31 and 31a, Lateral caudal filament of last instar female nymph, showing the subimago filament within. 32, 32a and 32b, Lateral caudal filament of female subimago showing lateral caudal filament of imago within. 33, 33a, Basal half of lateral caudal filament of adult female. 34, 34a, 34b and 34c, Basal portion of lateral caudal filament of adult male.*

In *Ephemera simulans* there is considerable metamorphosis during development, involving the antennae, caudal filaments, external genitalia, wing pads, eyes, mouth parts, gills and the number of setae and hairs.

The antennal segments are added one at each instar through most of the nymphal life and the caudal filaments add segments, in the following way,—one-half in the second instar, one in the third instar, one in the fourth instar, two in the fifth instar, three in the sixth instar, four in the seventh and in all subsequent nymphal instars. The twelve distal segments are dropped in the subimago. The mouth parts show considerable metamorphosis as shown by the appearance and rapid development of the mandibular tusks, beginning about the sixth and lasting to about the twentieth instar, after which the tusk increases at approximately the same rate as the body as a whole. The rostrum in its development keeps pace with the mandibular tusks. The gills make their appearance at the second instar as uniramous appendages, and increase in complexity and size during development. The internal or secondary ramus appears at the fifth or sixth instar, and develops rapidly, never, however, reaching the length nor the complexity of the primary ramus. The wing pads make their appearance at about the twelfth from the last instar, and grow out rapidly. The genitalia are not apparent as early as the wing pads, but are visible soon after, the claspers as rounded elevations on the posterior border of the ninth sternum, and the penes as rounded vesicles on the intersegmental membrane behind the ninth sternum.

Stenonema canadense Walker

The full-grown nymph of this species is well described by Clemens (1915).

The eggs are of a reddish color, and when shed into water adhere in a mass held together by a gelatinous material, and also by threads in skeins as described by Morgan (1913).

First instar, (Fig. 6,–1, 1a, 1b, 1c). Head as long as broad, posterior angles squarish; anterior border somewhat truncate; antenna with two basal segments and a flagellum in which one basal segment is differentiated and provided with an apical crown of spines, the remainder of the flagellum tapering and faintly marked off in pseudo- or sub-segments; compound eyes about the same size as lateral ocelli. The mouth parts were dissected out and are shown in Fig. 6,–1a, 1b, 1c (mandible, maxilla and labium); mandibles with cardinal teeth directed medially; molar surface not developed and there is an indication of a suture or joint across the mandible; maxilla and labium both unmodified and very different from the same structures in the full-grown nymph (Clemens, 1915).

In the thorax is the reddish, opaque mass of the yolk-containing enteron. Legs long and spindly, terminating in a long, scimitar-shaped, non-pectinate tarsal claw more than half the length of the tarsus; on each femur in the distal third a single posterior stout seta. Three subequal caudal filaments of which the telson or median is the longest; each filament composed of three well formed basal segments, each provided apically with a crown of spines,

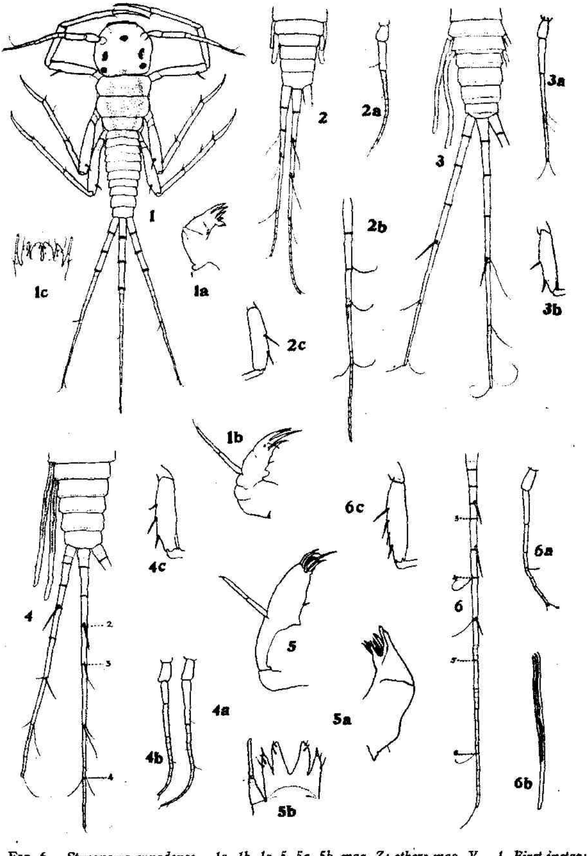

FIG. 6. *Stenonema canadense. 1a, 1b, 1c, 5, 5a, 5b, mag. Z; others mag. Y. 1, First instar; 1a, mandible; 1b, maxilla; 1c, labium. 2, Second instar; 2a, antenna; 2b, caudal filament, showing next instar within; 2c, femur. 3, Third instar, 3a, antenna; 3b, femur. 4, Fourth instar; 4a, antenna; 4b, antenna; 4c, femur. 5, Fifth instar, maxilla; 5a, mandible; 5b, labium. 6, Sixth instar, caudal filament; 6a, antenna; 6b, sixth gill; 6c, femur.*

TABLE II

DIAGNOSTIC CHARACTERS OF EARLY AND LATE INSTARS OF *Stenonema canadense*

Instar	Length in mm.			Segments in ant. flagellum, excluding terminal piece	Apparent no. of segments in antenna	No. of segs. in caudal filament, excluding terminal piece	Setae on posterior border of femur	Gills
	No. of individuals	Average	Lower and upper limits					
1	(20)	0.50	(0 45–0.55)	1		3	1	Absent
2	(28)	0.54	(0.47–0.59)	1½		4	2	On Segs. 5 and 6
3	(23)	0.62	(0.57–0.68)	2		5	2	On Segs. 5 and 6
4	(5)	0.69	(0.64–0.75)	3		7	3	On Segs. 5 and 6
5	(2)	0.72		3		9	3	On Segs. 5 and 6
6	(4)	0.81	(0.76–0.85)	4	3	11	4	On Segs. 5 and 6
7						14		On Segs. 5 and 6
?8				6	5	18	7	On Segs. 1-7.
9						22		On Segs. 1-7.
10				6	5	26 (app. 24)		On Segs. 1-7.
11				7		30 (app. 28)	17	On Segs. 1-7.
12 to about 32								

	Length of mesothoracic wing pad of male, mm.	Distance between wing pads at base, mm.	Segments in male claspers	Length of male claspers
Octult	0.19			visible
Septult	0.32	0.37		visible
Sextult	0.36	0.48		visible
Quintult	0.50	0.50	1 segment	visible
Quartult	0.69	0.38	2 segments	visible
Tertiult	0.91	0.45	2 segments	0.16 mm.
Penult	1.16	0.58	2 (and indistinct basal)	0.26 mm.
Last	1.95	0.32	3 segments	0.30 mm.

The length of the nymph is taken from the front of the head to the end of the abdomen, excluding the length of the caudal filaments; the number of individuals and the upper and lower limits of the measurements for each instar appear in brackets. As with Ephemera the two basal antennal segments are omitted in the count; but in this case the terminal portion of the antennal flagellum has also been omitted. Similarly in the count of segments in the caudal filaments the terminal undifferentiated portion has been excluded from the count. The setae on the femur refer to the posterior series only. Owing to fusion of segments basally in the antennae and caudal filaments the true number cannot be made out. The apparent number in these cases is also listed.

and a tapering flagellum divided indistinctly into about twenty subsegments; about half way along this undifferentiated portion of the flagellum, but slightly proximad, a hair which probably marks the position of what will be the apex of the next segment to be formed. Gills absent, but in some individuals the postero-lateral angles of Segments 5 and 6 project more than the others owing, probably, to the formation of gills below the cuticle, which will appear at the next ecdysis.

Second instar, (Fig. 6,–2, 2a, 2b and 2c). The antennae have changed slightly; the small subsegments of the flagellum adjacent to the segment already formed in the first instar have shortened and fused, or in other words, another segment has been partially differentiated; antenna has lengthened by a corresponding amount. Gills on Segments 5 and 6, only, and measure from $1\frac{3}{4}$–$2\frac{1}{4}$ times the length of an abdominal segment. A fourth segment has been differentiated in the flagellum of each caudal filament and the filaments have been increased in length; distally in Segments 2, 3 and 4, a single long, fine hair. Fig. 6,–2b shows one caudal filament of this instar inside which the new filament of the next instar can be seen. A long stout seta is discernible on this filament, beneath the cuticle at the junction of Segments 3 and 4 of the old filament.

Third instar, (Fig. 6,–3, 3a and 3b). Antenna now with two segments basally in the flagellum, *i.e.*, the segment which was being differentiated in the last instar is now formed and has elongated, although there are still vestiges of the former subsegments of which it is composed. Gills present on Segments 5 and 6 only, but they have elongated so that they measure from five to six times the length of a typical abdominal segment. Apically in Segment 4 of the caudal filament a stout seta which was mentioned above in the description of the second instar.

Fourth instar, (Fig. 6,–4, 4a, 4b and 4c). Two conditions of the antennae were found. The commoner shown in Fig. 6,–4a had a very long basal segment in the flagellum, with a thickened base which is probably the fusion of two segments. The other condition Fig. 6,–4b, had a much shorter basal segment in the flagellum. Probably one segment has been added to those formed in the third instar.

Gills present on Segments 5 and 6 only, but longer than in the previous instar, measuring from seven to eight times the length of a typical abdominal segment; caudal filaments with seven segments differentiated basally, of which Segments 1 and 2 are rather intimately fused and shortened, so that the separation is not very easily made out in some individuals; distally in Segment 4 the stout seta mentioned above, and a somewhat weaker seta present apically in Segment 6. Two segments have been added this time in contrast to the single segment added in the previous instars.

Fifth instar, (Fig. 6,–5, 5a and 5b). Gills present on Segments 5 and 6 only, and each about seven times as long as a typical abdominal segment.

In Fig. 6,–5, 5a and 5b, the mouth parts are shown greatly enlarged. These show some change since the first instar. Terminal lobes of the labium longer; molar process of the mandibles more developed. At this instar the mouth parts are still very different from those of the last instar, as will be seen by referring to Clemens' figures of the full-grown nymph (Clemens, 1915). At the stage in which about thirteen segments are recognizable in the antennae, however, the mouth parts are very similar to those of the full-grown nymph, so that the change in these parts must be rather abrupt and occur early in the life of the nymph.

Sixth instar, (Fig. 6,–6, 6a, 6b and 6c). Caudal filaments now with eleven segments differentiated, so that two new segments have been added distal to those already formed in the previous instar.

In the sixth instar there is, as in the fifth, a stout seta apically on Segment 4 of the caudal filaments, and also one present apically on Segments 6 and 8; apically in Segment 11 a long, thin hair. In Fig. 6,–6, the portion of the filament which has been differentiated at each ecdysis is indicated.

In the seventh instar three segments have been added to those formed in the sixth instar. At every subsequent ecdysis four segments are added to the caudal filaments.

The change into the eighth instar seems to be a very critical stage in the life history of this species. This is the stage at which the gills make their appearance on Segments 1 to 4 and 7. Only one stage was taken which appears to be the eighth instar. The stages beyond the eighth were not reared from the egg, but collected in the stream.

?Eighth instar, (Fig. 7,–7, 7a, 7b). Gills now present on all segments from 1 to 7 inclusive; on Segments 1 to 4 as short, rounded appendages, on 5 and 6 linear as in the fifth instar, and on 7, linear gills in length equivalent to the eighth abdominal segment. The caudal filaments were broken off in this specimen.

Tenth instar, (Fig. 7,–8, 8a, 8b and 8c). Gills of Segments 1 and 2 shown enlarged in Fig. 7,–8a; Gill 7 (Fig. 7,–8c) about twice as long as in the preceding instar. In the caudal filaments there are about 24 segments apparent, with stout bristles present apically in Segments 6, 8, 10, 12, 16 and 20; seta of Segment 4 has been lost. The regions of the filament which were differentiated at each instar are enumerated in Fig. 7,–8.

Eleventh instar, (Fig. 7,–9, 9a. 9b). Gill 7 has increased in length so that it is nearly as long as Gills 5 and 6. The segments of the caudal filament formed at each instar are shown in the figure.

The femora are provided with about ten long setae and about seven shorter intercalary setae (Fig. 7,–9b).

?Twelfth instar, (Fig. 7,–10, 10a, 10b, 10c, 10d, 10e, 10f, 10g, and 10h). The number of this instar could not be determined with certainty. Anterior gills show an increase in length over the previous instar, and are shown under greater magnification; Gill 2 longer than others of the anterior series; Gills 5, 6 and 7 long and linear. Antennae with seven segments differentiated in the flagellum.

Fourteenth instar, (Fig. 7,–11 and 11a, 11b and 11c). At this stage the anterior gills have grown out to a fair size and are lamellate. As yet there is no appearance of the tuft of filaments at the bases of the gills. The posterior gills, 5 to 7 inclusive, are still long and linear, but their length has decreased in relation to that of the nymph.

The mesothorax is shown in Fig. 7,–11a. The postero-lateral angles, at which point the wing pads later develop, are quite evenly rounded.

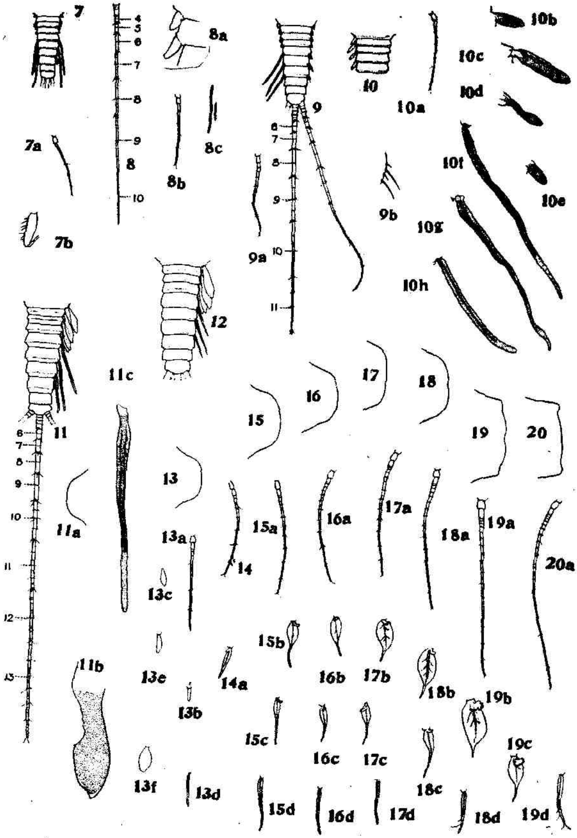

FIG. 7. *Stenonema canadense. 8a, 9b, 10b–10h, 11b, 11c, mag. Y; others mag. X. 7, ?Eighth instar, gills; 7a, antenna; 7b, femur. 8, Tenth instar, caudal filament; 8b, antenna; 8c, sixth and seventh gills; 8a, Gills 1 and 2. 9, Eleventh instar; 9a, antenna; 9b, part of femur. 10, Twelfth instar (anterior four gills); 10a, antenna; 10b–10h, Gills 1–7 under mag. Y. 11, Fourteenth instar; 11a, mesothorax; 11b, 11c, Gills 1 and 6. 12, Fifteenth instar. 13, Mesothorax; 13a, antenna; 13b, regenerating fifth gill; 13c, fourth gill; 13d, seventh gill; 13e, sixth gill; 13f, second gill. 14, Antenna; 14a, fifth gill. 15, Mesothorax; 15a, antenna; 15b, fifth gill; 15c, sixth gill; 15d, seventh gill 16, Mesothorax; 16a, antenna; 16b, fifth gill; 16c, sixth gill; 16d, seventh gill. 17, Mesothorax; 17a, antenna; 17b, fifth gill; 17c, sixth gill; 17d, seventh gill. 18, Mesothorax; 18a, antenna; 18b, fifth gill; 18c, sixth gill; 18d, seventh gill. 19, Mesothorax; 19a, antenna, 19b, fifth gill; 19c, sixth gill; 19d, seventh gill. 20, Mesothorax; 20a, antenna.*

?Fifteenth instar, Fig. 7,–12. Anterior gills larger and posterior ones have become smaller and more lanceolate; Gill 5 more affected by this process than 6, and in subsequent instars the former keeps ahead of the latter in its metamorphosis.

Beyond this point in the life history it was not possible to determine the number of the instar accurately from the material which was collected until nearer the end of nymphal life, when the last seven instars were fairly accurately determined.

Fig. 7,–13 to 20a, shew the changes taking place in certain structures in an incomplete series of nymphs from a stage (13a) in which there were eight or nine segments differentiated in the antennae up to a stage (20a) in which nineteen segments were differentiated. Gradual change at each ecdysis is evident in the posterior border of the mesonotum, with the incidence of wing pad formation at about the middle of the series as a gradual prolongation of the postero-lateral angles of the notum. Gill 7 shows little change throughout the series, but Gills 5 and 7 show considerable change from the linear type found in Gills 5 to 7 in early life to the lamellate type of the anterior segments.

At the stage in which there are eight segments differentiated in the antennal flagellum, the ventral lobe of the fifth and sixth gills has made its appearance and during subsequent instars this lobe becomes more complex until finally it is a tuft of filaments. The basal part of these gills becomes more and more expanded and the terminal part shortens until finally Gills 5 and 6 from their very different appearance early in development come to resemble the anterior gills. Gill 7 does not change noticeably and never develops the tuft of filaments at the base.

Fig. 8,–21, 21a, 21b, 21c and 21d show parts of a later instar in which there are twenty segments differentiated in the antennal flagellum. The mesothoracic wing pads are more prominent than in previous instars. Gills 5, 6 and 7 are shown greatly enlarged in Fig. 8,–21b, 21c and 21d.

Fig. 8,–22, 22a, illustrate a later instar in which 21 or 22 segments are differentiated in the antennal flagellum.

Fig. 8,–23, 23a, 23b, 23c and 23d are of a later instar in which 23 or 24 segments are differentiated in the antennal flagellum. The mesothorax is shown in Fig. 8,–23. In Fig. 8,–23b and 23c are shown Gills 5 and 6 which are now much like the anterior gills and have lost the terminal filamentous portion.

Fig. 8,–24 and 24a are of a still later stage, in which about 25 segments are present in the antennal flagellum. The wing pads measure about .1 mm. in length. In this instar the developing external genitalia are not apparent, but in the next instar they can be made out so that it is possible from here on to separate male and female individuals on this character. With the wing pads and external genitalia developing through the next instars it was possible by an examination of a number of individuals to determine what are believed to be the last eight instars.

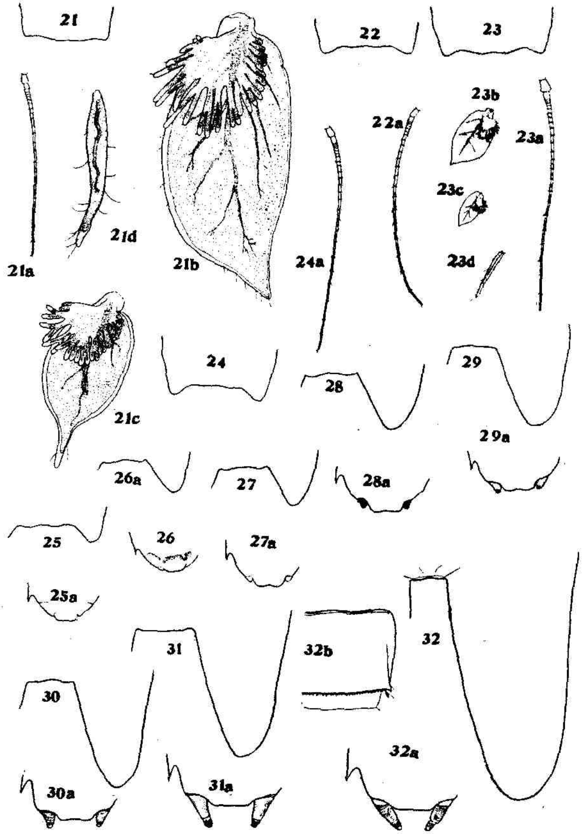

FIG. 8. *Stenonema canadense.* *21b, c, d, mag. Y; others mag. X.* *21, Mesothorax; 21a, antenna; 21b, fifth gill; 21c, sixth gill; 21d, seventh gill.* *22, Mesothorax; 22a, antenna.* *23, Mesothorax; 23a, antenna; 23b, fifth gill; 23c, sixth gill; 23d, seventh gill.* *24, Mesothorax; 24a, antenna.* *25, Mesothoracic wing pad; 25a, developing male genitalia.* *26a, Mesothoracic wing pad; 26, male genitalia.* *27, Mesothoracic wing pad; 27a, male genitalia.* *28, Mesothoracic wing pad; 28a, male genitalia.* *29, Mesothoracic wing pad; 29a, male genitalia.* *30, Mesothoracic wing pad; 30a, male genitalia;* *31, Mesothoracic wing pad; 31a, male genitalia.* *32, Mesothoracic wing pad; 32a, male genitalia; 32b, seventh abdominal tergum.*

Octult instar, (Fig. 8,–25, 25a). The developing male claspers may be distinguished at this stage as small indentations on the postero-lateral margins of the sternum. There is a suture separating the posterior portion of the sternum from the anterior sternite proper, which suggests that this posterior part of the sternum may be the fused appendages of this segment, part of which will develop into the claspers and the medial part be associated with the penes.

Septult instar, (Fig. 8,–26, 26a). Male claspers have not increased much in size and are rather difficult to distinguish as in the previous instar.

Sextult instar (Fig. 8,–27, 27a). Male claspers not very distinct, but visible as rounded projections on the lateral borders of the sternum; their bases visible; they extend less than half way from their bases to the posterior border of the ninth sternum.

Quintult instar, (Fig. 8,–28, 28a). Male claspers composed of one segment only, darkly pigmented with a pale tip, extending only slightly more than half way from their bases to posterior border of ninth sternum.

Quartult instar, (Fig. 8,–29, 29a). Male claspers of two segments; small distal segment pigmented to tip; claspers reach the level of posterior border of ninth sternum or project very slightly beyond it.

Tertiult instar, (Fig. 8,–30, 30a). Apical segment of clasper pigmented in proximal half and unpigmented distally; basal segment pigmented, the pigment being distributed in annuli.

Penult instar, (Fig. 8,–31, 31a). Male claspers about .26 mm. in length, composed of two distinct segments and an indistinctly marked off basal segment; middle segment of clasper long and smooth; apical segment short and darkly pigmented distally to the apex.

Last instar, (Fig. 8,–32, 32a, 32b). In Fig. 8,–32b is shown the tergum of the seventh abdominal segment. The females are larger as a rule, having a wing pad length of about 2.2 mm.

The changes taking place in the caudal filaments from nymph to subimago and from subimago to imago are illustrated in Fig. 9,–33 to 36. In Fig. 9,–33, 33a, 33b, of the caudal filament of penultimate nymphal instar, there are about 122 segments, formed as indicated in about 40 instars. In the subimago and imago filament there are decidedly fewer segments, about 70 in the imago. This reduction may come about as in *Ephemera*, by the dropping of distal segments and probably also by the loss of a few basal segments by fusion and obliteration of the sutures.

There is considerable metamorphosis of structure during nymphal development in *Stenonema canadense*. Some structures, such as antennae and caudal filaments, develop continuously with the growth of the nymph, maintaining their length with little increase in relation to the size of the nymph. These structures, particularly the caudal filaments, are the most sensitive indicators of change, and record every ecdysis as far as can be made out. Other structures, such as the mouth parts, wing pads and external genitalia,

develop during a particular period of the nymphal life and during this time change greatly relative to the changes in the nymph as a whole.

The mouth parts change radically during the early instars, but by about the twelfth have a form similar to that of the full-grown nymph. The gills show definite metamorphosis apart from an increase in size. The gills of Segments 5 and 6 appear at the second instar and those of Segments 1 to 4 and 7 do not make their appearance until about the eighth instar. Gills 1 to 4 grow directly into flat lamellae, but Gill 7 becomes long and linear and similar to Gills 5 and 6. The latter pair gradually metamorphose until they are similar to Gills 1 to 4, but Gill 7 remains as a single lanceolate appendage known as the vestigial gill. Gills 1 to 6 develop a tuft of filaments ventrally near the base.

The wing pads make their appearance about the fifteenth from the last instar, and grow back at first rather slowly, then by gradually accelerated growth. The external male genitalia are first distinguishable in about the eighth from the last instar. The claspers in this instar are slightly rounded projections from the ninth sternum and the anlage of the penes lies between them. The caudal filaments at hatching have three segments differentiated basally and a terminal undifferentiated termina part to the flagellum. In the second instar there is one further segment differentiated in the caudal filaments, making four in addition to the terminal piece, which, however, is only about half as long as in the first instar. Apparently there has been very little growth during the first instar. In the third instar there are five segments differentiated, and the fourth bears a stout seta apically. In the fourth instar there are seven segments differentiated, show'ng that two segments are being added at each instar instead of one as in the earlier instars. In subsequent instars two segments are added up to the seventh, when three segments are added. In the eighth instar and in all subsequent instars, four segments apparently are added at each ecdysis except in the subimaginal and imaginal instars. The individual new segments added at each instar are of approximately equal length and volume in the early stages, so that change in the rate of growth is indicated by the manner in which the segments are added, the supplemented segments increasing in number in the ratio of 1 : 1 : 2 : 2 : 2 : 3 : 4 : 4, from the first to the ninth instars.

Curiously it is at the eighth instar that the gills make their appearance on Segments 1 to 4 and 7, a fact which may be more than coincidental since the increase in rate of growth probably implies a greater oxygen requirement. Also, as mentioned above, this stage is a very critical one in the life of the nymph as indicated by failure to rear them beyond this stage in conditions under which they incubated well and passed through the first stages successfully.

In Fig. 9,–33 a complete lateral caudal filament of a female individual of the next to last nymphal instar is shown. The portions of the filament laid down at each instar are indicated and a count of these regions beginning at the apex reveals that there are 39 instars recorded which, with the last,

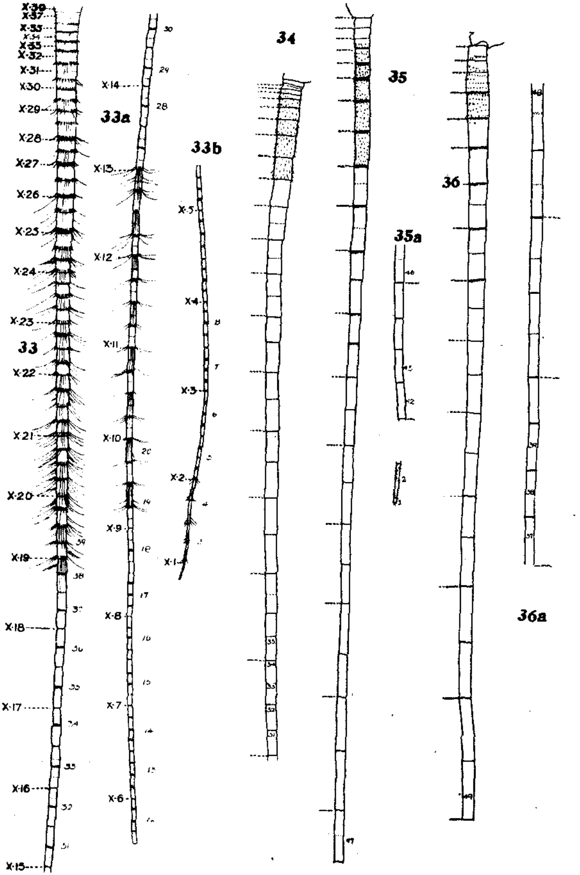

FIG. 9. *Stenonema canadense. All mag. X.* 33, 33a, 33b, *Complete lateral caudal filament (cercus) of a female nymph in the penultimate instar.* 34, *Lateral caudal filament of subimaginal female.* 35, 35a, *Lateral filament of adult female;* 36, 36a, *Lateral filament of adult male.*

make 40. Unfortunately, this is not the full number of instars represented during the nymphal period. Fusion of segments at the base and their almost entire obliteration results in a smaller number than was actually differentiated during development. There are probably between 40 and 45 instars in this species. Of these, 35 have been illustrated showing how they differ morphologically, and obvious gaps in the sequence noted.

Ephemerella subvaria McDunnough

An account of the full-grown nymph of this recently described species is given by McDunnough (1931). This was a common species in the streams studied by the author and females were frequently taken ovipositing. The eggs are extruded in a spherical mass of a brownish yellow color, which is dropped in flight into the water of the rapids.

The eggs are ovoid in shape, finely reticulate, and measure about .2 by .15 mm. They are stuck end to end by a mass of very elastic gelatinous material so that when the string of eggs is stretched this material pulls out into a thin elastic thread. Sometimes the strings branch and anastamose, three eggs instead of two being held together by one mass of the elastic material.

First instar, Fig. 12,–8. Three individuals measured on an average .495 mm. in length, exclusive of the caudal filaments. The head is somewhat triangular with the apex directed more ventrally than in *S. canadense;* antennae with two distinct basal segments, a very short proximal and a longer distal one, and a flagellum in which three segments are distinguishable including the apical portion; in front of each compound eye a curved hair.

Each of the femora bears distally a curved hair or fine seta; tarsal claws pointed and with two rows of pectinations converging on the large apical claw.

Caudal filaments relatively short, equalling about one third the length of the body; three well developed basal segments and an apical undifferentiated part which shows in some cases an indication of weak joints; median caudal filament slightly longer than the lateral ones; gills absent.

Isonychia bicolor Walker

The full-grown nymph of this species is described by Needham (1905) under the name *Chirotenetes albomanicatus* Needh. which is placed as a synonym by McDunnough.

The eggs, (Fig. 12,–2), are spherical in shape with a diameter of .25 mm. They are extruded from the female in the form of a spherical mass of an olive green color, in which form they are dropped on the surface of the water during flight, and quickly disperse.

First instar, (Fig. 12,–1). The average length of six individuals was .71 mm. with a range of from .67–.75 mm. Gills have not made their appearance

and the yolk is still present as the opaque body shown in stipple. The compound eyes are less prominent than the lateral ocelli and are situated directly behind them.

Antennae with two basal segments and a tapering, flexible flagellum in which one proximal segment has been differentiated; tarsal claws pectinate; femora with only one rather stout distal seta on the posterior border and a somewhat weaker dorsal one; three caudal filaments each consisting of three well developed proximal segments, each of which bears a conspicuous crown of spines and a terminal undifferentiated part bearing a few hairs.

Second instar, (Fig. 12,–3, 3a, 3b). Gills have not appeared; antennae with no increase in number of segments; still but one segment differentiated proximally in the flagellum; the terminal portion of the flagellum is not, however, tapered as in the first instar, but more cylindrical with a slight constriction in the middle; setae on posterior border of femora increased to two on fore femur and three on hind femur, on which they are well developed, the middle one being the longest; caudal filaments show a definite change in the addition of one segment, making four well differentiated segments proximally; this change may easily be overlooked, however, since the crown of spines on the proximal segment has been so reduced that it is visible only with the closest observation. The new distal segment (Segment 4), has been added from the undifferentiated distal part of the filament of the first instar; basal Segments 1 and 2 intimately fused together, the crown on Segment 1 being almost obliterated. In the next instar it is very probable that the separation between 1 and 2 will not be apparent, the fusion being complete, and so on through the life of the nymph, segments are formed apically and proximal ones lose their identity in the process of fusing to form a strong rigid base for the filament.

One individual in this stage had a portion of the exuvia of the first instar attached, showing plainly the single spine distally in the third femur, and also the three-segmented condition of the bases of the caudal filaments. The fusion of Segments 1 and 2, which makes it impossible to distinguish them in some individuals and very difficult in others, probably led to Lubbock's conclusion that growth takes place by an elongation of the basal segment and its ultimate division into two. In describing the second instar of *Cloeon dimidiatum*, Lubbock, (1864) writes: "The two tails have increased to a length of 25/200, and consist of twenty segments." (It should be mentioned that this large number of segments is due to the fact that Lubbock includes the small subsegments of the flagellum in his count.) "Here again as in the antennae almost the whole change has taken place in one segment, which however, is in the present case the basal one. The remainder are almost exactly as they were before. As already mentioned the basal segment was in the first stage 5/800 in length; in the present it has divided into two segments which, taken together, are 9/800 and we see therefore that almost the whole increase of length is in this one part."

In another section of the present paper the addition of segments has been discussed and it is evident that segments are added from the distal end to those already present, and not by a division of segments which have been differentiated. The basal segments are not becoming shorter by being subdivided, but by thickening and fusing together.

Fig. 12,–4, 4a, 4b show a later instar whose number was not determined owing to the absence of antennae and caudal filaments which had been broken off. From its size (1.20 mm.) and the number of setae on the femur, it is probably about the fourth. At this stage the gills are making their appearance as rounded projections from the postero-lateral angles of the segments. They are slightly longer on Segments 4, 5 and 6, but probably appear simultaneously on all segments as was the case with *Ephemera simulans.* The claws are pectinate.

These early stages were reared from the egg in water from the stream standing in an open jar. Eggs from several females were set to incubate on July 6, 1931. On July 24 some had hatched although none were out on July 21. Four first instar individuals were taken in a sample from the jar on July 24. On July 28 a sample was taken which contained one individual in the first instar and twenty in the second instar. On August 11 one individual was removed from the jar and this one was in about the fourth instar. No others were seen on this date. On August 15 one first instar nymph was taken. Development in this species is apparently very rapid. The temperature of the water in the jar was of course higher than is normal for hatching of this species.

Epeorus humeralis Morgan

The full-grown nymph is described by Dr. Anna Morgan (1911).

The eggs (Fig. 10,–1a) which are of a reddish color, are extruded from the female in two ribbon-like masses but quickly disperse on touching the water. The length is about .20 mm. and the width .12 mm. They are not provided with special organs of attachment.

First instar, (Fig. 10,–1). The average length of six nymphs was .40 mm., the limits being about .33 to .42 mm. The head broader than long and convex in front; the compound eyes (the pigmented portion of them at least) smaller than the lateral ocelli; antennae with two basal segments and a flagellum in which one segment is differentiated basally; portion of flagellum distal to this segment divided into small faintly demarcated subsegments as mentioned above in the description of *S. canadense*. The opaquely reddish mass of the enteron is visible through the integument of the thorax. This is shown as a stippled area in the figure.

Abdomen without gills; lateral caudal filaments (cerci) with three segments differentiated at the base, each with a crown of spines apically; remaining portion of caudal filament tapered and divided into small subsegments; median caudal filament with one basal segment and a distal three-segmented flagellum.

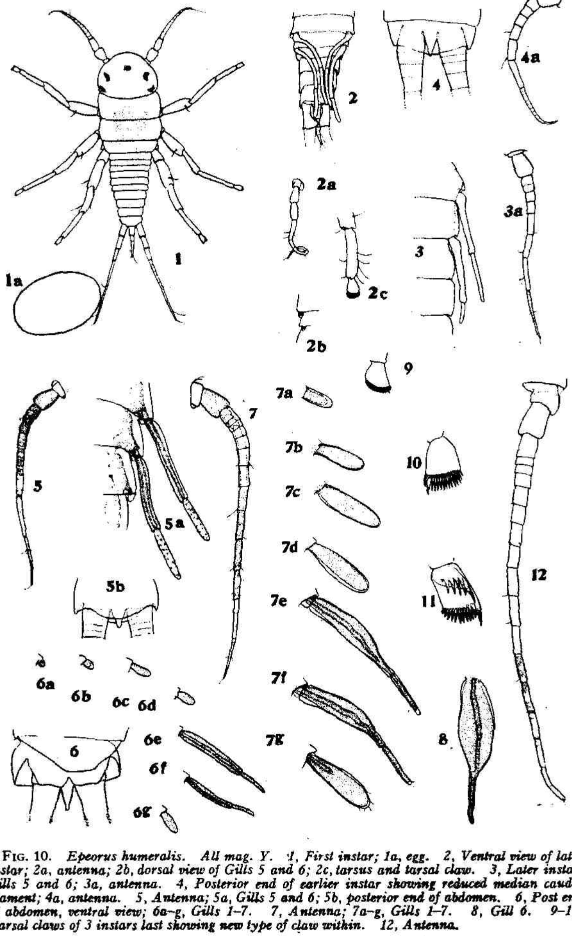

FIG. 10. *Epeorus humeralis. All mag. Y. 1, First instar; 1a, egg. 2, Ventral view of later instar; 2a, antenna; 2b, dorsal view of Gills 5 and 6; 2c, tarsus and tarsal claw. 3, Later instar, Gills 5 and 6; 3a, antenna. 4, Posterior end of earlier instar showing reduced median caudal filament; 4a, antenna. 5, Antenna; 5a, Gills 5 and 6; 5b, posterior end of abdomen. 6, Post end of abdomen, ventral view; 6a–g, Gills 1–7. 7, Antenna; 7a–g, Gills 1–7. 8, Gill 6. 9–11, Tarsal claws of 3 instars last showing new type of claw within. 12, Antenna.*

A later instar (Fig. 10,–2, 2a, 2b and 2c) shows two well developed segments in the antennal flagellum and measures .73 mm. Gills are present on Segments 5 and 6 only, long and filamentous, directed ventrally, and divided into two segments, a long basal and a shorter distal one. The fact that the gills are directed ventrally makes them very inconspicuous, and it was some time before they were noticed.

Lateral caudal filaments thick and bulbous at the base, narrowing somewhat suddenly into the main filament which had dropped off in most individuals; median caudal filament present as a short three-segmented appendage; tarsal claws are particularly interesting, consisting of a series of about 15 curved hooks of nearly equal size. One of these at one end of the series is, however, stouter. In development it is this stouter claw which will become the tarsal claw proper and those of the others which remain will become the pectinations along one side of the main claw.

In a later instar (Fig. 10,–4, 4a) in which there are six segments in the antennal flagellum, the terminal segments of the median caudal filament are dropped, leaving a short conical projection in its place. A later stage (Fig. 10,–3, 3a) is shown in which the antennal flagellum has about seven segments. The gills are present on Segments 5 and 6, but are now directed laterad rather than ventrad.

In the stage in which eight segments are formed in the antenna (Fig. 10,–5, 5a and 5b), the gills are present on 5 and 6, as two-segmented structures, and the gills of some of the other segments have appeared as mere rounded elevations on one or two of the other segments. The relative sizes of the gills (Fig. 10,–6, 6a–g) are shown on all segments in a nymph measuring 1.9 mm. The gills of 5 and 6 are still two-segmented. The gills are also present now on Segments 1, 2, 3, 4 and 7 as short, rounded appendages. The gills are shown (Fig. 10,–7, 7a–g) at the stage in which the antennae have 12 segments differentiated. Those on Segments 1-4 and 7 are becoming lamellate but are still rather thick and the tracheae are not apparent. The tracheae are showing in the basal segments of Gills 5 and 6. The latter pair are broadening at the base. Length of nymph is 2.33 mm. Gill 5 is shown (Fig. 10,–8) in a later stage in which the nymph measured 3.4 mm. Gills 5 and 6 are now very similar to the other gills, except that they are formed of two segments, the distal one of which is not becoming lamellate. The antennal flagellum at this stage is composed of 16 well developed segments.

The terminal segment of Gills 5 and 6 is dropped after the stage in which the antennae contain 22 well developed segments in the flagellum and is absent from the later instars.

Fig. 11,–13, 13a–d illustrate some of the characters of this stage which measured 4.13 mm. Gill 6 still has a vestige of the terminal part which is dropped at the next shedding. The wing pads have been developing through several ecdyses. The tarsal claw has changed, the number of hooks or claws having been reduced from about fifteen in the stage illustrated on Fig. 10,–10, to five of which one is much longer than the others and more curved. The smaller ones form now the pectinations of the larger claw.

Fig. 11,–14 and 14a–e show some structures of the last instar. Fig. 11,–14c is of the tarsal claw of the third leg, which now consists of one great, curved claw bearing four smaller straight ones as pectinations. Gills 1, 5 and 7 are shown in ventral view. Of particular interest is the chitinous thickening around the outer margin of the gills armed with numerous thickly set short stiff spines directed downward and backward, which have been described by Morgan (1913). When the nymph is facing upstream into the current, as they usually do, these spines are in contact with the stone on which it is resting, thus firmly anchoring the nymph to the rock. It should be noticed that the limpet-like adaptation is not as perfectly developed in *Epeorus* as in *Iron pleuralis*, since *Epeorus* lacks the long anterior process which is present on Gill 1, of *Iron*, and also the fold in the seventh gill of the latter, which would allow the gills of the seventh segment to fit more closely together. The appearance of the genitalia of female and male which have been developing during the later instars is shown in Fig. 11,–14d and 14e respectively.

Curiously enough the abrupt transformation of the tarsal claws illustrated in Fig. 10,–11, coincides with the point in development at which the gills have grown out far enough to come in contact with the stone on which the nymph is resting, thus aiding in anchoring it. It also coincides roughly with the time in the life of the nymphs at which there is a marked tendency to drop out of the rapids into the somewhat quieter water below. This was determined in an ecological study of this species, which is presented in another paper, (Ide, 1935).

The nymphs of this species occur commonly along with those of *Iron pleuralis*, the next species, but in the younger stages can be readily distinguished from the latter in the field by the presence of a light yellow area on the anterior part of the dorsum of the abdomen, which is contrasted with the dark greenish color of the rest of the nymph. As a young nymph *Iron pleuralis* is uniformly light brown.

Iron pleuralis Banks

The full-grown nymph of this species has already been described, with some notes on its habits (Ide, 1930).

This species follows *Epeorus humeralis* very closely in its post-embryonic development. The earliest stage collected in the stream is one (Fig. 11,–15, 15a and 15b), in which the antenna has but three well formed segments in the flagellum, and which measures about 1.10 mm. in length. The lateral caudal filaments have 19 segments and the median filament is vestigial having but two segments present. Gills are present on Segments 5 and 6 only, where they are very long and filamentous, and composed of two segments, a distal short and a basal long one.

At the stage (Fig. 11,–16, 16a–f) in which there are six segments in the antennal flagellum, the gills on Segments 1, 2, 3, 4 and 7 have appeared as minute bud-like processes. The gills on Segments 5 and 6 are composed of two segments, but have become somewhat stouter. The median caudal filament

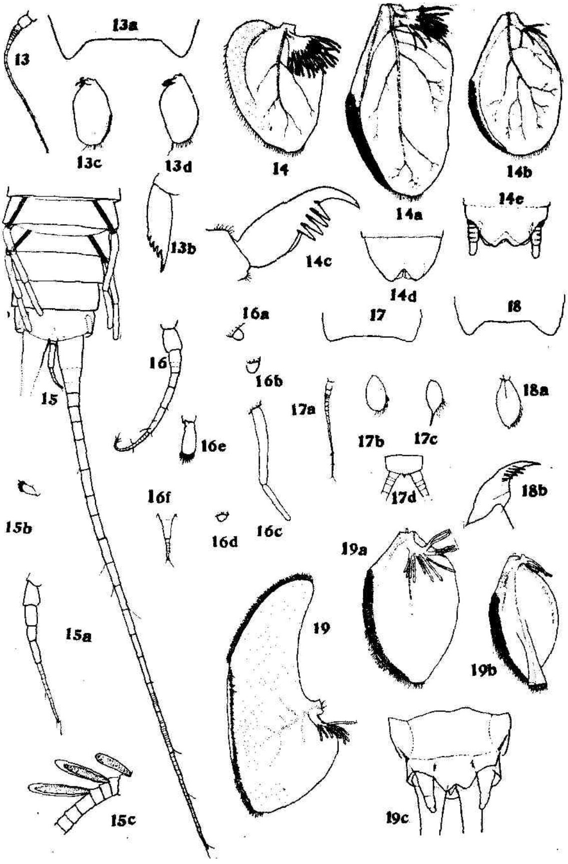

FIG. 11. *Epeorus humeralis.* *13b, 14c, mag. Y. others mag. X.* *13, Antenna; 13a, mesothoracic wing pads; 13b, tarsal claw; 13c, fourth gill of right side; 13d, sixth gill of right side. 14, Last nymphal instar Gill 1, ventral view; 14a, Gill 5; ventral view; 14b, Gill 7, ventral view; 14c, tarsal claw; 14d, posterior end of female, ventral view; 14e, male genitalia.*

Iron pleuralis. *15–16f, and 18b, mag. Y; 17–19c mag. X.* *15, Nymph about seventh instar; 15a, antenna; 15b, tarsal claw; 15c, parasitic fungus protruding from the anus. 16, Antenna; 16a, b, c, d, Gills 1, 2, 5, 7; 16e, tarsal claw; 16f, median caudal filament. 17, Mesothorax; 17a, antenna; 17b, Gill 3; 17c, Gill 6; 17d, posterior end of abdomen, showing reduced median caudal filament. 18, Mesothorax; 18a, Gill 6; 18b, tarsal claw. 19, Last nymphal instar, Gill 1; 19a, Gill 5; 19b, Gill 7; 19c, male genitalia, ventral view.*

is similar to the last described stage except that Segment 2 has become relatively shorter and more conical. The tarsal claws of *Iron pleuralis* differ from those of *Epeorus* in that the most strongly developed claw is near the middle of the series of claws rather than at one end, as was the case in the latter. Length in this stage is about 1.48 mm.

A later stage is illustrated (Fig. 11,–17, 17a–d) in which the specimen is 2.90 mm. long and the antenna contains 14 well developed segments in the flagellum, showing that the nymph has passed through several moults since the last described instar. The gills are now flat lamellae as shown in Fig. 11,–17b, 17c for gills of Segments 3 and 6 respectively. The terminal segment is present on Gills 5 and 6 as a narrow, cylindrical process. The distal segments of the median caudal filament have now been dropped (Fig. 11,–17d). About two instars later, when the antennae have about seventeen segments, the terminal segments of Gills 5 and 6 are dropped (Fig. 11,–18, 18a, 18b). The sixth gill is shown in Fig. 11,–18a, with a slight tubercle at its apex, the remnant of the former distal segment. At this stage the gills are all rather similar, although they have originated at different times in development.

From now on the metamorphosis is chiefly in the growing wing pads, the external genitalia, the antennae and caudal setae. In Fig. 11,–19, 19a–c, the detail of Gills 1, 5 and 7 of the last instar is given. The most notable feature is the anterior extension of Gill 1 which passes beneath the thorax ventrally, where it nearly meets the corresponding gill of the opposite side. In the gill of the seventh segment there is a fold medially directed in such a way that the gills of both sides nearly meet below the abdomen. All the gills, by this arrangement, form a sucking disc, the water pressure keeping the gills firmly pressed against the rock. Along the lateral borders, where the gills meet the rock, are series of short spines directed backwards, which form an efficient anchor, as Morgan has pointed out for *Iron fragilis* (1913).

In this species as in *Epeorus* there has been a considerable metamorphosis in gills and tarsal claws. In the younger stages the gills are not developed as organs of retention and so the whole load comes on the tarsal claws, which are numerous, small and arranged in a V-shaped line at right angles to the force so that they provide a maximum grip. The nymph is so small that it is only the minute irregularities of the surface of stones which are of use to it for retention, and in order to grip these irregularities there must be many, closely set, tarsal claws. As the nymph grows in size the gills become important as gripping organs and the claws change, until ultimately there is one large tarsal claw with pectinations along the inner border. These pectinations are derived from some of the smaller tarsal claws present in the early stages of development. The claws of the larger nymphs are probably used more for crawling than for clinging to the surface of the stone, and the irregularities against which they pry are not of the order which are useful earlier in the life history but much larger ones which are naturally much farther apart. A series of small tarsal claws in the form of a rake probably would not be as efficient now as the single pectinate claw. This is an interesting example

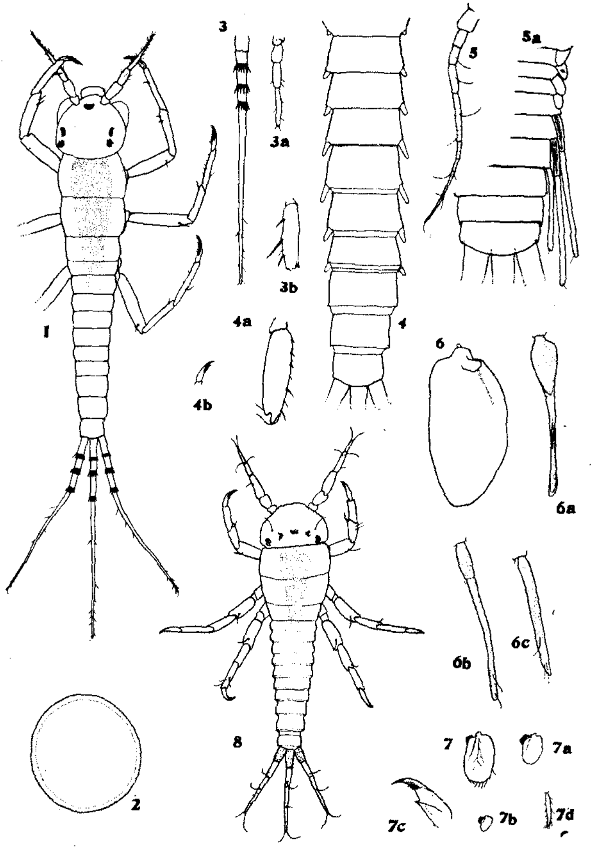

FIG. 12. *Isonychia bicolor. All mag. Y. 1, First instar. 2, Egg. 3, Second instar, lateral caudal filament; 3a, antenna; 3b, hind femur. 4, About fourth instar, showing gills; 4a, hind femur; 4b, tarsal claw.*

Stenonema fuscum. 5–6c, 7c, mag. Y; 7, 7a, b, 7d, mag. X. 5, Antenna; 5a, gills; 6, Third gill, ventral aspect; 6a, fifth gill; 6b, sixth gill; 6c, seventh gill. 7, Third gill; 7a, fifth gill; 7b, sixth gill; 7d, seventh gill; 7c, tarsal claw of foreleg.

Ephemerella subvaria. Mag. Y. 8, First instar.

of an organism radically changing its relation to its environment by growth. As it grows larger it has to adjust itself differently to the same flow of water, so that it is rather the relation of the environment to the organism which is important as a cause in metamorphosis than change in the environment itself.

This change in the tarsal claws is probably quite abrupt as was the case with *Epeorus* described above. An individual was not found which showed the change, but intermediate steps in the process were not seen in considerable material examined.

Heptagenia pulla Clemens

The last nymphal stage is described and figured by Clemens (1915). The first stage was not found in the present study.

A nymph (Fig. 13,–1, 1a, 1b) measuring 1.6 mm. in length showed the following points. The antennae have four well developed segments in the flagellum. The three caudal filaments, of which the central one is slightly longer than the others, are composed of 23 segments, the basal three of which are fused so as to be indistinguishable. Strong setae are present apically in Segments 6, 8, 10, 12 and 16. On Segment 14 there is a stiff hair only, so that probably Segments 15, 16, 17 and 18 were added at one ecdysis; this is probably the ninth or tenth instar.

Gills are present on Segments 2, 3, 4, 5, 6 and 7. On Segment 2 the gill is a mere bud; on Segments 3, 4 and 7 the gills are unsegmented filaments, a little longer than their respective segments. On Segments 5 and 6 the gills are about twice as long as on 4 and 7, and composed of two segments. By analogy with *Epeorus* and *Iron* the gills presumably appear on Segments 5 and 6 at an earlier ecdysis than on the other segments.

During subsequent development the gills assume a lamellate form and the tuft appears at the base of the gill, as shown for an intermediate stage in Fig. 13,–4, of Gill 5. All gills from 1 to 7 are now very similar except that 5 and 6 retain the terminal segment. This terminal segment is present on Gills 5 and 6 in the penultimate instar (Fig. 13,–3, 3a–c) and sometimes even in the last instar, as a mere vestige which has been generally overlooked in the published figures of this species.

Heptagenia hebe

A full-grown nymph was figured and described by Clemens (1915) under name *Ecdyonurus maculipennis*. This species was not reared in connection with the present study, but nymphal material was collected from the stones in the streams.

The earliest stage found (Fig. 13,–5 and 5a) shows the antennae with four well developed segments in the flagellum. The three caudal filaments are similar except for the slightly greater length of the median one. They consist of 16 well marked segments with strong setae distally on Segments 4, 6, 8, 10 and 12, and a terminal flagellum. It is probably about the seventh instar. Gills are present as long filaments on Segments 4, 5, 6 and 7, those on 5 and 6

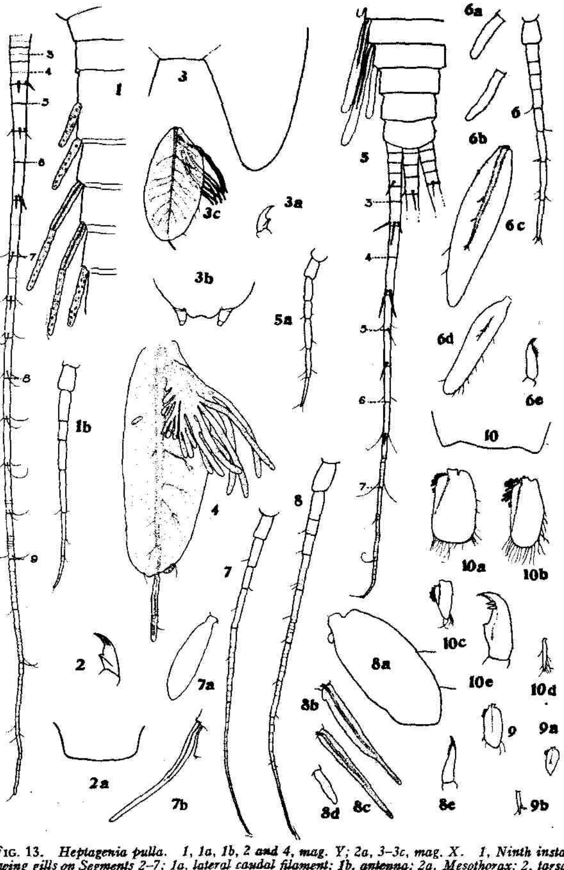

FIG. 13. *Heptagenia pulla*. *1, 1a, 1b, 2 and 4, mag. Y; 2a, 3–3c, mag. X. 1, Ninth instar showing gills on Segments 2–7; 1a, lateral caudal filament; 1b, antenna; 2a, Mesothorax; 2, tarsal claw; 3, Penultimate instar, mesothoracic wing pads; 3a, tarsal claw; 3b, male genitalia; 3c, Gill 5. 4, Fifth gill.*

Heptagenia hebe. *5–6e mag. Y. 5, Seventh instar; 5a, antenna. 6, Antenna; 6a, 6b, 6c, 6d, Gills 1, 2, 5, 7 respectively; 6e, tarsal claw.*

Stenonema tripunctatum. *7–8e and 10e mag. Y; 9–10d, mag. X. 7, Antenna; 7a, third gill; 7b, fifth and sixth gills. 8, Antenna; 8a, second gill; 8b, c, d, fifth, sixth, seventh gills; 8e, tarsal claw. 9, Gill 5; 9a, Gill 6; 9b, Gill 7; 10, Mesothorax; 10a, Gill 3; 10b, Gill 5; 10c, Gill 6; 10d, Gill 7; 10e, tarsal claw.*

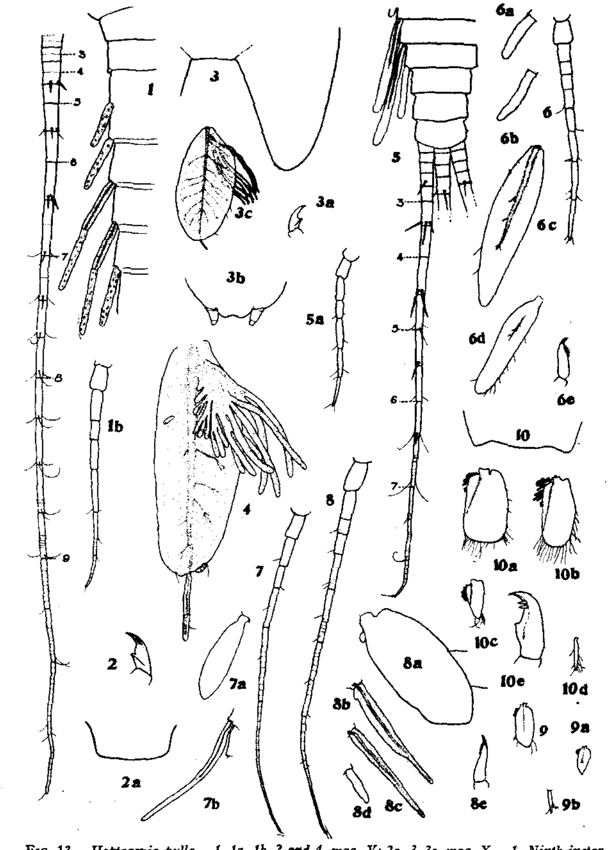

FIG. 13. *Heptagenia pulla. 1, 1a, 1b, 2* **and** *4, mag. Y; 2a, 3–3c, mag. X. 1, Ninth instar showing gills on Segments 2–7; 1a, lateral caudal filament; 1b, antenna; 2a, Mesothorax; 2, tarsal claw; 3, Penultimate instar, mesothoracic wing pads; 3a, tarsal claw; 3b, male genitalia; 3c, Gill 5. 4, Fifth gill.*

Heptagenia hebe. 5–6e mag. Y. 5, Seventh instar; 5a, antenna. 6, Antenna; 6a, 6b, 6c, 6d, Gills 1, 2, 5, 7 respectively; 6e, tarsal claw.

Stenonema tripunctatum. 7–8e and 10e mag. Y; 9–10d, mag. X. 7, Antenna; 7a, third gill; 7b, fifth and sixth gills. 8, Antenna; 8a, second gill; 8b, c, d, fifth, sixth, seventh gills; 8e, tarsal claw. 9, Gill 5; 9a, Gill 6; 9b, Gill 7; 10, Mesothorax; 10a, Gill 3; 10b, Gill 5; 10c, Gill 6; 10d, Gill 7; 10e, tarsal claw.

being about twice the length and of greater thickness than those on 4 and 7, but not segmented as in *Heptagenia pulla*. Here again, as in other cases referred to earlier, it seems extremely probable that there were gills present on Segments 5 and 6 before there were any on the other segments.

In a later stage (Fig. 13,–6, 6a–e) in which the antennae are composed of seven well differentiated segments, the gills are further advanced in development, and present on Segments 1 to 7. On Segments 1 and 2 they are short, on Segments 3 and 7 about twice as long as on 1 and 2, and on 4 they are slightly longer than on 3 and 7. On 5 and 6 they are longer than on any of the other segments but lack the terminal segment found in *Heptagenia pulla*. This seems to be a significant difference between the two groups in the genus *Heptagenia*, but of course may not prove to be a group character.

As development proceeds the gills grow broader, develop the tuft of filaments at the base, but otherwise do not change much and are very similar in form to one another. The gills on 5 and 6 keep ahead of the others in size, however, and the others retain their differences in relative length. It seems probable that in this form both segments of Gills 5 and 6 of *Heptagenia pulla* are represented in the corresponding unsegmented gills, and that the whole length of the gill has entered into the formation of the lamella. This would account for their relatively greater length in the ultimate nymphal instar.

In development of gills this species probably follows closely *Ecdyonurus forcipulus* Koll (see Grós, 1923).

The tarsal claws are pectinate as shown for the stage in which the antenna is of seven segments as shown in Fig. 13,–6e.

Stenonema tripunctatum Banks

The full-grown nymph is well described by Clemens (1915). In the earliest instar found the antennal flagellum contains four well differentiated segments (Fig. 13,–7). The anterior gills were lamellate and similar to the third of the left side which is figured in Fig. 13,–7a. Gills 5 and 6 (Fig. 13,–7b) are long and filamentous and similar to one another, and the gills of 7 are lacking entirely. It seems likely that in *Stenonema tripunctatum*, as in *S. canadense*, the gills of Segments 5 and 6 appear before those of any of the other segments.

The next stage found was the instar in which the antennal flagellum is composed of six well differentiated segments Fig. 13,–8. At this stage the gills on 1 to 4 are lamellate with the tuft of filaments beginning to form at the base. Gill 2 is shown in Fig. 13,–8a. The gills on 5 and 6 (Fig. 13,–8b and 8c) are long and filamentous, that of 5 being somewhat broader at the base and constricted toward the distal extremity. Gill 7 (Fig. 13,–8d) is not linear, but rather short, differing in this respect from *S. canadense*. The tarsal claw (Fig. 13,–8e) has the middle spine very much developed and the others arranged on each side of it as two rows of pectinations.

In a later stage the antennae have about 13 segments differentiated in the flagellum. The gills of this instar on Segments 5, 6 and 7 are illustrated in Fig. 13,–9, 9a and 9b. Gill 7 is lanceolate and lacks the tuft of filaments

at the base. Gill 6 is lamellate and has dropped the terminal portion whose former position is marked by a small tubercle at the apex of the gill. Gill 5 is lamellate and shows no sign of the linear tip; it has metamorphosed so that it is similar to the anterior gills. Gills 5 and 6 both have the tuft of filaments at the base developed at this stage.

In a still later stage (Fig. 13,–10, 10a–e), in which the antennae have about 19 well developed segments in the flagellum the gills are very similar to those of the full-grown nymphs. The main change now takes place in the greater size of the gills and the greater complexity of the tuft of filaments at the base. In this stage the wing pads (Fig. 13,–10) have been developing through three or four instars and the claws (Fig. 13,–10e) have changed somewhat, the change being mainly in the reduction in the number of pectinations. In one series there are two, and in the series of the opposite side, but one angular projection which may be a different structure entirely from the claws which form the pectination. Gill 7 has not changed its form. Gill 6 has lost the vestige of the terminal part, is triangular in shape and shows a greater development of the tuft of filaments at the base. Gills 3 and 5 are similar to one another and have a truncate posterior border.

Stenonema fuscum Clemens

The full-grown nymph is described by Clemens (1915) and also by McDunnough (1933). This form was not studied in great detail, but three stages are figured, which throw some light on the development of gills in this group. At the stage (Fig. 12,–5, 5a) in which the antennal flagellum contains four segments proximally, and the nymph is about 1 mm. in length, gills are present on Segments 1 to 4 as short rounded projections varying slightly in length, but in general being about equal to the length of the corresponding abdominal segment. Gill 3 is slightly longer than the other gills of the anterior series; Gill 7 is long and unsegmented, similar to the gills of the same segment in *S. canadense*. The gills on 5 and 6 are similar to each other and are divided into a short basal segment and a terminal segment which is nearly three times as long as the basal one. These gills are more like those of *Iron*, *Epeorus* and *Heptagenia pulla* than those of *Stenonema canadense* in this respect. Their greater length and the presence of the trachea in the basal segment suggest that these gills appeared earlier than the other gills, probably at the second instar. The tarsal claws are similar to those of *S. tripunctatum* in possessing two rows of pectinations, one on either side of the central claw.

Fig. 12,–6, 6a–c, taken from an individual 1.7 mm. long in which nine segments were differentiated in the antennal flagellum, forecast the probable fate of each segment of the fifth and sixth gills. The basal portion has become expanded (6a), the content has been constricted off at the intersegmental region, so that at the next instar the distal segment will be dropped altogether and the basal segment will form the ultimate gill lamella as in *Epeorus*. Gill 7 is unsegmented and remains practically the same throughout development, and in the full-grown nymph is referred to as a vestigial gill.

Fig. 12,–6 shows the tuft of gill filaments forming at the base of the lamella of Gill 3. Gills 1–4 are all lamellate and similar to Gill 3, although varying somewhat in size. In Fig. 12,–7, 7a–d are shown Gills 3, 5, 6 and 7 at a later stage (antennae 16 segments) when Gills 5 and 6 have lost their distal segments and are similar to Gills 1 to 4. Gill 7 retains its simple and lanceolate character.

Leptophlebia debilis Walker

The full-grown nymph of this species is described by the author (Ide, 1930).

Some of the younger stages of this species were found. In the stage in which there are eleven segments in the antennal flagellum, the gills (Fig. 4,–27 to 29) are very long, slender filaments, showing the beginning of the inner or upper ramus of the gill. This ramus, which later becomes nearly as long as the primary ramus, is at this stage a mere bud at the base of the gill. A branch of the trachea runs into it. Undoubtedly in an earlier stage the gills would be simple lanceolate filaments without the side branch. A European genus, *Paraleptophlebia*, has been figured in this stage (Lestage, 1916).

Discussion and Conclusions

A study of the life histories of these nymphs has brought out some points which will be discussed under five headings:

(a) Origin of segments in the antennae and caudal filaments.

(b) Variation in the number of caudal filaments present in mayflies.

(c) Metamorphosis of claws and mouth parts.

(d) Appearance and metamorphosis of gills.

(e) Metamorphosis in general.

Origin of Segments in the Antennae and Caudal Filaments

Both antennae and caudal filaments show some points regarding the formation and addition of segments during growth, which should be noted. The antennae and filaments roughly maintain their length relative to the size of the instar, with a few exceptions (see *Baetis*).

In the very small nymphs a very flexible flagellum is satisfactory, but as the nymph grows in size the basal part of the flagellum must become more solid and rigid for some distance, the intermediate part must be flexible, accomplished by the production of segments or joints which allow of movement and yet are rigid enough to give support. Rigidity and increase in size at the base of the flagellum is accomplished by the compression of the segments until they are much thicker than long, and then by fusion to form a rigid base. This gives the appearance of a growing point at the base of the flagellum, the segments originating by a splitting of the original segments into two or four parts, as suggested by Lubbock, (1864 and 1867). The development of the flagellum of the antennae and caudal filaments in *S. canadense* and others demonstrates that the segments do not arise in this way, but rather by a differentiation of segments from the proximal end of the

unsegmented flagellum, the new segments being added distal to those formed in the previous instar. The clearest evidence for this is found in an examination and comparison of the terminal unsegmented part and the adjacent segments of the antennae in early instars of *S. canadense*. The terminal part will be seen to be divided into minute subsegments of about equal volume (Fig. 6,–2a). As we pass proximad a place is found where these subsegments quite suddenly become noticeably shorter and appear to be more immovably united to one another. This point is usually roughly shown by the appearance of one or several hairs a little proximad of it. About ten of these subsegments make up one segment and what is being examined then is a segment in the process of formation. Next to this region proximally is a well formed segment in which, however, the original subsegments of which it is composed are clearly distinguishable. Examination of more proximal segments in later instars shows that the vestiges of these subsegments are lost entirely nearer the base.

The origin of segments in the caudal filaments takes place in the same way except that here the segmentation is still more complex. The small subsegments of the terminal part fuse in numbers to form a segment. Then at the base of the filaments in later stages the segments themselves become indistinguishably fused with one another to form a rigid base. Further, the segments formed are not uniformly definite, even at the time of their appearance. In *S. canadense* one segment makes its appearance at the first ecdysis. At the next ecdysis one segment is added and a stout seta appears distally in Segment 4. At the next ecdysis two segments are added, the proximal one (Segment 6) bearing apically a stout seta similar to that on Segment 4. The separation between the fifth and sixth segments is not nearly as well defined as that between 6 and 7, and thus, as development proceeds, Segments 5 and 6 will soon become fused so as to be indistinguishable and this fusion will not take place between 6 and 7 till a later period in development. Segments 13 to 16 are produced also at one shedding; now the joint between 12 and 13 and that between 16 and 17 are each much more prominent than the joint between 14 and 15, and the joint between 14 and 15 is in turn more definite than the joint between 13 and 14 and that between 15 and 16. Thus, as development goes on and these segments begin to fuse and enter into the basal part of the filament, the junctions between 13 and 14, and between 15 and 16 will disappear first; then the joint between 14 and 15, and finally the joint between 12 and 13 and that between 16 and 17.

The limit of the segment is determined in the undifferentiated terminal part of the flagellum. Usually these joints form in a definite way in regard to the position of the hairs and setae, so that these structures are in the distal part of the segment. Sometimes, however, something goes wrong and the segment limit or joint does not fall at the right place, and the hairs are not present distally in the proper segment. For example in the third instar of *S. canadense* there is, with very few exceptions, a strong seta distally in Segment 4 of the caudal filaments. In an occasional specimen, however, (and

in these it is usually in but one of the filaments) the seta is absent from Segment 4 but is present distally in 5. In this case, however, Segment 4 is much shorter than the normal, and Segment 5 is also rather short, showing that the joint had occurred at the wrong place. Even so, the circlet of spines forms distally in both 4 and 5. From this it would appear that the position of the setae in the caudal filaments is more fundamental than the position of the segments. In this connection Lubbock noted that the dark bar on the caudal filaments of *Cloeon dimidiatum* retained its position in the distal third throughout the nymphal period, in spite of great changes in the position of the segments.

In the caudal filament (Fig. 5,–30, 30a) of a full-grown nymph of *Ephemera*, there are a number of intimately fused segments at the base, but many of them are still distinguishable. As the segments are examined distally they become more distinct and then show a division into two, which is at first very indistinct. The suture dividing these secondary segments forms a sinuate line rather than the straight line between the original or primary segments. Still further out on the tail the primary segments show division into four, or a subdivision of the two halves, also by sinuate lines. At first these sutures, which might be termed tertiary sutures, are indistinct, but further out they become very distinct so that each primary segment is delimited by a straight suture from adjacent primary segments and divided into two secondary segments by a sinuate line, and these secondary segments are each divided into two tertiary segments by a sinuate line or suture. In development, each primary segment represents the addition to the caudal filament at one moult. The basal primary segments are undivided; a few adjacent segments are divided into two secondary segments; those formed subsequent to the eighth instar are composed of four tertiary segments. The caudal filament (Fig. 5,–30, 30a) does not show all the segments formed owing to loss through fusion at the base. And furthermore, as pointed out above, in the process of fusion and disappearance of the intersegmental sutures, the tertiary ones disappear first, then the secondary and finally the primary. If all the segments remained separate and distinguishable it would be possible to count the number of primary segments in the filament and thus have the number of instars through which the nymph has passed. This number, together with the well known subimago and adult instars, would be the full number passed through by the individual. In the subimago instar there is apparently a freeing again of at least some of the segments fused during nymphal life. In Fig. 5,–30 to 34c, the corresponding segments in full-grown nymph, subimago, and male and female imagos are similarly designated. The determination of these homologies was made possible by the finding of nymphs about to emerge, which showed the enclosed segments of the subimaginal caudal filaments, and also by the fact that the imaginal segments were apparent within the subimaginal cuticle. The segments, it will be seen, correspond very well with those of the previous stage, the main difference being an increase in length of the segments. In the case of *Ephemera*, the pigmentation at the joints helped greatly in working

out the homologies in the last three instars. Fairly well out on the tail, as mentioned above, each primary segment is composed of four tertiary segments, these being separated not by straight lines as are the joints between the primary segments, but by sinuate lines. The subimaginal tail developing within the nymphal tail near the end of the last nymphal instar fortunately shows a difference in pigmentation at the junction of the segments. This indicates that the segments correspond. Between the primary segments there is very little pigmentation, while between the secondary and tertiary segments there is very dark pigmentation, resulting in the middle third of the caudal filament in the formation of a light band followed by three dark bands, then another light and three dark bands, and so on. This plan can be followed well out on the tail, a few segments only, near the tip, not showing distinctly the grouping into four by the pigmentation. Towards the base the dark rings between the tertiary segments disappear first and then finally those between the secondary segments. There is a narrow constriction at the base of the subimago caudal filament, designated X, which can be fairly accurately placed and so serve as a starting point in a count of the segments.

In *Stenonema* also the segments are apparently added in fours at each ecdysis through most of the nymphal life. Here the segments are also nicely grouped in fours not by the difference in character of the joints themselves as was the case with *Ephemera*, but by the presence of setae at the apices of the segments and also somewhat by the pigmentation. In the basal part the primary segments from the fourth on are armed in the middle with very stout spines in a single row. Farther out the spines disappear, but they have been present far enough out on the tail to show that the method of addition of the segments is apparently the same as in *Ephemera simulans*. In the basal third of the filament the apices of the primary segments are not armed with thick, stout setae, but with longer, thinner ones and possess a band of pigment. Following out the tail to the region of the tertiary segments it is seen that the pigmented band disappears from the region between the primary segments and is now found on the line dividing the tertiary segments. After the disappearance of the stout setae in the middle of the primary segments distally in the caudal filament there is nothing to distinguish them, so that a primary segment is represented by two secondary segments which were added at one ecdysis and are similar to one another as far as could be ascertained.

Each of these secondary segments is divided by a suture into two tertiary segments with darkened bands between. Basally the segments are fused together so that first the tertiary joints disappear, leaving two secondary segments only to each primary, and then the secondary joints disappear, leaving only the primary joints, which eventually disappear also.

In *Ephemera* the change in the caudal filaments from the subimago to the imago stage was the most difficult to understand. The imaginal filament was much longer than the corresponding subimaginal filament, and it was surprising to find that in spite of this there were fewer segments present and

that the lengthening had been caused by the elongation of segments. At first it was concluded that the decrease in the number of segments had been caused by fusion of segments at the base, but an individual which was about to change into the subimago showed that this was not the case. In this individual (Fig. 5,–31a) the reduction was caused by the dropping of the twelve distal segments of the nymphal filament. Other specimens of this species examined later showed the same phenomenon. The process is the same as that taking place in the antennae between the last nymphal instar and the subimago as described by Lubbock (1867) for *Cloeon dimidiatum*.

Variation in the Number of Caudal Filaments Present in Mayflies

The number of tails present in mayflies has always been an interesting question. Several different conditions seem to exist.

1. Nymphs hatch with three caudal filaments of about equal length, and retain these throughout nymphal and adult life, *e.g.*, *Ephemera*, *Ephemerella*.

2. Nymphs hatch with three caudal filaments of about equal length, retain these throughout nymphal life, and lose the middle tail in the subimaginal and adult stages, *e.g.*, *Stenonema*, *Heptagenia*.

3. At hatching, there are two well developed lateral filaments and a reduced median, one which is further reduced in early nymphal life, and remains so throughout the later stages, including the subimago and adult, *e.g.*, *Iron* and *Epeorus*.

4. Nymphs hatch with two well developed lateral filaments, but no median one. The median one grows out during nymphal life though not attaining the length of the lateral ones. In the subimago and imago the median filament is dropped and the lateral ones retained, *e.g.*, *Baetis posticatus* (Murphy 1922), *Baetis vagans* and *Cloeon dimidiatum* (Lubbock).

5. There is perhaps another condition too in which there are only two caudal filaments at hatching (the two lateral ones or cerci), the third or median filament not being present or making its appearance during subsequent stages including the subimago and adult. *Pseudocloeon* seems to illustrate this type but since first stage nymphs have not been examined it may turn out that *Pseudocloeon carolina* has a reduced median filament which is dropped early in development as was the case with *Iron* and *Epeorus*.

The two-tailed condition in nymphs seems to be associated with life in very rapid water (*Iron*, *Epeorus* and *Pseudocloeon*) where obviously the tails are not used for swimming and therefore the addition of a middle tail could be of little advantage.

The lateral caudal filaments are generally conceded to be the cerci, appendages of the eleventh segment, and the median caudal filament the greatly elongated telson.

The evidence available seems to indicate that the three-tailed condition is primitive within the order Ephemeroptera, the two-tailed condition having arisen secondarily by the dropping of the median filament. The great

majority of species have three caudal filaments in the nymphal instars, some of which drop the median caudal filament in the subimago and adult, and others retain it. Some of the nymphs which have but two tails present have a vestigial median filament in the early instars and many adults which apparently have but two filaments have a rudiment of the median present. Apparently the median filament is a very variable structure, being present in the adults of some species and absent in the adults of other species, even in the same family. In order to consider the two-tailed condition primitive, the assumption would have to be made that the median tail had been independently developed in several families, which is extremely improbable. The logical conclusion is that the three-tailed condition is primitive and that mayflies had three-tailed insects as ancestors.

Metamorphosis of Claws and Mouth Parts

In *Stenonema* the molar surface of the mandible is relatively undeveloped at the time of hatching, and the other mouth parts (Fig. 6,–1a–c) also differ greatly from their final condition in the half-grown or full-grown nymphs. The canine teeth on the mandibles of each side are directed medially so that they probably function in ingestion. Later these canine teeth are directed anteriorly and probably do not take part in ingestion in the same way as formerly, that function being taken on by the molar surface. This suggests that the canine teeth function as early larval organs.

An examination of stomach content was not made, but, assuming that the young nymph eats the same organisms and materials as the older nymph, we would expect that a different mechanism would be required in a small and large nymph in order to capture effectively and take in the same organisms. The relation of food organism and nymph has altered not by any change in the former, although this may occur also, but merely by the increase in size of the nymph which necessitates morphological changes to meet the altered environmental relation thus created.

A series of tarsal claws of *Epeorus humeralis* is shown (Fig. 10,–2c, 9, 10, 11, Fig. 11,–13b and 14c), illustrating the metamorphosis which takes place in these parts. There is a gradual increase in the number of claws up to the stage illustrated by Fig. 10,–11, at which time there is a reduction in the number of claws as indicated by the new claw developing within. The new claw has changed its form very abruptly. There is from now on one great curved claw with some of the other claws present as a row of pectinations on the great claw. This abrupt change in the type of tarsal claw coincides with a probable change in function of the claw as described under *Epeorus humeralis*.

The nymph throughout its life cycle must be adapted to hold itself in position in a strong current of water. Obviously the very small nymph will hold to very minute irregularities on the surface of the stones. The many-pectinated or many-clawed last tarsal segments of the early stage nymphs are adapted to clinging to such a surface. With growth these claws increase in number up to the stage shown in Fig. 10,–11, when there is a sudden meta-

morphosis of the claws, to form the type shown in Fig. 11,–13b, 14c, with one claw greatly elongated and hooked and the four others present as pectinations along its lower surface. The nymph by this stage is much larger than at hatching (about 6 mm. long), so that the minute irregularities on the surface of the rock will no longer provide a suitable hold, but irregularities of a larger order, and therefore less in number relative to the nymph's immediate environment, will be used. For this purpose the new type of claw will be much more satisfactory since it will hook around these larger irregularities and projections. This sudden metamorphosis of the claws also coincides with the time at which the gills reach the substratum and develop along their lower edge combs of posteriorly directed setae, which function in holding the nymph in place in the current of water. It is just at this time in their life that the nymphs of *Epeorus humeralis* drop down-stream out of the very rapidly flowing water into the more slowly flowing water below the rapids (Ide 1935).

Appearance and Metamorphosis of the Gills

The gills appear as prolongations from the postero-lateral angles of the segments. In *Ephemera* and *Isonychia* they appear simultaneously on Segments 1 to 7 inclusive. In *Stenonema canadense*, *Epeorus humeralis* and *Iron pleuralis* the gills of Segments 5 and 6 appear several instars earlier than those on 1, 2, 3, 4 and 7 and this is probably true for *Heptagenia pulla*, *H. hebe*, *Stenonema tripunctatum* and *Stenonema fuscum*. The gills of 5 and 6 in these forms grow out as long filaments which later metamorphose into lamellae similar to the anterior gills which have developed into lamellae directly, never having the filamentous form. Gill 7 in some forms becomes filamentous, *e.g.*, *Stenonema canadense*, or may develop as a lamellate plate as in *Heptagenia*. The metamorphosis of Gills 5 and 6 from filaments to lamellae may take place in two ways. If these gills are composed of two segments as in *Epeorus humeralis*, *Iron pleuralis*, *Heptagenia pulla* and *Stenonema fuscum* the change involves only the basal segment which becomes expanded and lamellate and the distal segment remains unchanged and is eventually dropped. If the gills are unsegmented, as in the case of *Heptagenia hebe*, *Stenonema canadense* and *Stenonema tripunctatum*, the whole gill enters into the formation of the lamella. In the former process the result is a gill with a rounded or truncate posterior border while in the latter the result may be a pointed gill as in *S. canadense*. All gills appear first as uniramous structures, but early in their growth most of them develop a secondary medial ramus which may take several forms. In *Ephemera* and *Leptophlebia* it grows out and becomes similar to the primary ramus though not attaining its length or complexity. In *Epeorus*, *Iron*, *Stenonema* and *Heptagenia* the secondary ramus takes the form of a plumose tuft of filaments. The secondary ramus may be absent from all the gills as in *Baetis*, or may be absent from one or more gills, *e.g.*, Gill 7 in *Stenonema* which remains uniramous throughout development.

The gills contain tracheae and some of them at least are provided with muscles which move them to and fro in the water. Fig. 11,–15 represents

a young nymph of ***Iron pleuralis*** in which muscles pass from a suture on the venter of the segment (line separating the zygosternum into two parts, the sternum and the limb bases) to the proximal part of the gill. This muscle does not continue into the gill even when the latter is a two-segmented structure. Durken (1907) has discussed the musculature of the tracheal gills of ephemerids and has come to the conclusion that the gills are dorsal or notal structures, although he does not homologize them with wings as some others have done. The more generally accepted view is that they represent abdominal legs (Snodgrass, 1931, and Spieth, 1933).

Metamorphosis in General

The number of instars in mayflies is very great compared with that in other insects, a phenomenon probably inherited from ancestors and retained because of the special requirements of this group. At each of the moults there is some increase in size of the nymph but the moult seems primarily for the purpose of changing the morphological structure. Although the nymph is increasing slowly in size, some structures such as mouth parts, wing pads, gills and genitalia are changing radically in size and complexity by differential growth rate of the parts. Some of this metamorphosis is merely concerned in perfecting the adjustment of nymphal structure to the changing conditions. Examples are the changes in mouth parts, eyes, gills and tarsal claws. Other metamorphosis is concerned with the development of such adult structures as the wings and external genitalia.

The cuticle of insects is very elastic so that it seems unlikely that so many moults could be concerned primarily with increase in size. Many other insects grow to much larger size with but four or five moults. The reason for the great number of moults seems to be in the constant adjustment which the nymph must make to its physical environment (*e.g.*, food, rapid flow of water). Every change in external structure, even to the addition of a seta or hair, can be accomplished only by a moulting of the old skin and the emerging of the new stage adapted to its brief duration as was each stage prior to it. A long resting period is precluded in these forms because of the necessity of their being maintained in a rapid current and actively feeding all the time. Consequently any large structure does not form completely at one moult, but requires several, as for example, in the formation of the mandibular tusks in *Ephemera*. Very abrupt changes are rather the exception than the rule in this group and most of these occur when the insect leaves the water, at which time the environment is radically changed. Examples of such changes are found in the development of turbinate eyes in some forms, in the great reduction in size of the antennae, often in the dropping or aborting of the median caudal filament or in the shortening of the filaments as was demonstrated for *Ephemera simulans*. There is a striking difference in the time required to develop a structure such as a caudal filament or an antenna and the time required to get rid of the same structure. By a process of differential growth, which probably follows closely the exponential curve, the structure is gradually developed through several instars but it may be eliminated in much less time, at one ecdysis.

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CYTOLOGY OF THE BLOOD OF NORMAL MINK AND RACCOON

I. MORPHOLOGY OF MINK'S BLOOD[1]

By Arnold H. Kennedy[2]

Abstract

The morphology and number of blood cells occurring in normal mink and raccoon have been determined in order to establish normal standards for various periods during the life of these animals. These standards are desirable for comparative purposes when determining the extent of pathological blood changes which may take place during the course of nutritional or parasitic diseases.

Normoblasts and poikilocytes were observed in mink's blood. Reticulocytes were also common. Forms of lymphocytes ranging from 6.5 by 6.0 μ to 11.3 by 9.5 μ are found. The monocytes range in size from 11.0 by 9.5 μ to 15.5 by 13.0 μ. In the neutrophile, band-formed nuclei are common, comprising from two to eight per cent of the total number. Their sizes range from 11.5 by 11.0 μ to 16.0 by 12.0 μ. Numerous, well defined, reddish-brown granules are found in the cytoplasm of the eosinophile. In the eosinophile also, the lobes of the nuclei are less lobulated and closer together than in the neutrophile. The eosinophile may range in size from 11.5 by 10.0 μ to 16.0 by 15.0 μ. The cytoplasm of the basophile contains from 22 to 65 large prominent granules, which stain dark blue to purple. Granules which are almost indiscernible are also present in the cytoplasm. The basophile ranges in size from 11.5 by 11.0 μ to 14.0 by 12.0 μ. Large masses of platelets ranging in size from 1.5 by 1.0 μ to 2.5 by 2.0 μ are often observed in smears from mink's blood.

Introduction

It was decided that the morphology and number of blood cells occurring in the normal mink and raccoon should be determined before attempting to record data obtained from blood examinations during the course of investigations of the nutrition and the parasites of these animals. This series of papers is based on data obtained from examinations of the blood of normal mink and raccoon. Each paper covers a portion of the work and the series contains descriptions of the cellular morphology and photomicrographs of each cellular element for comparative purposes. Statistical tables have been prepared showing the blood counts for the various age groups of each species.

No references relating to the blood of these animals could be found in the literature. In determining the extent of pathological blood changes, normal standards for various periods during the animal's life are desirable for comparative purposes. It is hoped, therefore, that the material contained in these papers may save some other worker a considerable amount of tedious work and serve as standards for the blood of normal mink and raccoon.

Materials and Methods

The animals used for these studies were selected from breeding stock and their progeny kept at the Ontario Government Experimental Fur Farm. Animals under a year old were bled at various times throughout the year. The data obtained were classified according to the sex and age of the animals.

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Contribution from the Ontario Government Experimental Fur Farm, Kirkfield, Ontario.
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Animals over a year old were considered as adults. The ages of adult mink varied from over one, to four years; the ages of adult raccoon from one to eight years. Blood counts taken from pregnant females or females suckling young were not included.

Blood examinations were not made on animals less than three weeks old because female mink and raccoon resent interference with their young and consequently may destroy them if they are handled at birth or soon after.

All animals were in good health and nutrition. No disease or parasites appeared among them during the course of the work. The housing arrangements and feeding and environmental conditions generally were uniform for each species, and were those accepted as being adequate for ranch-raised mink and raccoon. Standardized equipment was used throughout the investigation.

Extraction of Blood

The mink were held in restraint by having an assistant grip them firmly around the neck with one hand and hold the foot from which the blood was to be extracted with the other. A small net and thick leather gloves were used in catching and handling the mink.

The raccoon, excepting those under two months old, were caught in a net, and a large funnel about two and a half feet long, twelve inches across at one end and tapering to about three inches at the other, was placed over the head. The raccoon was forced into the funnel, from which a hind foot could readily be drawn out and held in position for bleeding without fear of the animal biting.

The most suitable place for extracting blood from the mink is similar to that described for the fox (1), *i.e.*, the blood vessel found in the integument between the toes.

The site found most suitable for obtaining blood from the raccoon is further up on the foot, between the metacarpal bones and close to one of them The metacarpal blood vessel is not visible but a plentiful supply of blood is readily obtained by puncturing the skin with a small sharp pointed scalpel (No. 11. Bard Parker Blade).

Before obtaining samples from these locations the fur was clipped with a pair of scissors and the area cleaned with alcohol. After the puncture was made the first few drops were rejected in order to avoid the local effects of manipulation and any possible contamination with loose tissue.

Enumeration of the Blood Cells

For the enumeration of the red blood cells, the blood was diluted 200 times in a standardized Trenner diluting pipette (1–101). Toisson's fluid was used throughout the investigation for diluting purposes. A haemocytometer certified by the United States Bureau of Standards was used for counting the red blood cells. This has Levy-Hausser counting chamber and improved Neubauer double ruling. The enumerations were based on the examination

of 100 squares, each 1/400 mm. long. These were composed of six groups of 16 and four additional squares. The groups were selected from the milled area of the counting chamber. No attempt was made to select any given group.

The average size of the red blood cells was determined by the use of the so-called halometer or eriometer, which gives a direct measurement of the red blood cell by the diffusion of light. The instrument was first described by Pryce (2) (second method).

The white blood corpuscles were counted in the standardized haemocytometer and sampled with a Trenner diluting pipette (1–20). The diluting fluid was 0.3% glacial acetic acid in aqueous solution. The white corpuscles covering one sq. mm. in each quarter of the milled area were counted. The proportions of the different types of white blood cells were determined by classification of 200 consecutive white cells from a blood film. Their respective numbers were then estimated from the total number found in a cubic millimetre of blood.

The Morphology of the Blood Elements

The technique used in making and staining the films for studying the size shape and appearance of the various corpuscles in detail and for making differential counts of the white blood cells was that described by Kennedy (1)

Morphology of Mink Blood

Red blood cells. The red blood cells when stained with Hasting's stain give a pink to rose-red color. The centre stains paler than the periphery owing to the biconcave formation of the cell. In young mink, the cells appear to stain a lighter color.

The average diameter of the red blood cells is 7.8 μ; the maximum size is 9.5 μ and the minimum 6.0 μ. Normoblasts were found in mink of all ages but were most frequently seen in mink three and four months old and were also quite common in the adult mink. One to six normoblasts have been recorded in an examination of 200 leucocytes.

Reticulocytes are common in mink blood; anisocytosis was observed in young suckling mink; poikilocytosis was observed in mink of all ages.

Lymphocytes (Plate I, Figs. 1 and 2). Large and small forms of lymphocytes are found in mink blood, but intermediate forms ranging in size from the small to the large make it impossible to separate them into two groups. The smaller forms are more numerous than the larger. Their nuclei, which are usually round, oval or slightly indented in shape, comprise the larger portion of the cell. A narrow band of cytoplasm may surround the nuclei or a small crescent-shaped portion may be visible. In the larger forms, the nuclei are round, oval, bean-shaped, or slightly lobed, situated in the centre or to one side of the cell, and are usually surrounded by a comparatively wide band of cytoplasm.

The nucleus of the smaller forms appears more compact and stains a dark blue to purple color. In the larger forms heavily staining nuclear masses are separated by pale narrow bands. The cytoplasm of the smaller lymphocytes stains a pale blue color while the cytoplasm of the larger forms stains more deeply and shows a clear gray-blue color.

The average size of the lymphocyte is 11.3 μ by 9.5 μ. The maximum size is 15.0 by 12.0 μ, and the minimum size is 6.5 by 6.0 μ.

Monocytes. (Plate I, Fig. 3). The nucleus may be bean-shaped, U-shaped or kidney-shaped, with the convex side usually facing the margin of the cell. The nucleus stains a dark blue color, with portions of the dark blue stained chromatic material being divided by bands staining a light greenish to gray-blue color, similar to that of the cytoplasm. The cytoplasm has a slightly roughened appearance and no granules were observed in it.

The average size of the monocyte is 13.0 by 10.0 μ. The maximum size of the monocyte is 15.5 by 13.0 μ and the minimum size is 11.0 by 9.5 μ.

Neutrophiles. (Plate I, Figs. 4 and 5). In appearance, the neutrophile of the mink is not unlike the neutrophile of the fox (1). The nuclei stain a deep blue to a purple color and are segmented, having from two to seven lobes, five being the average. The lobes may be quite widely separated and connected by a narrow filament. Portions of the nuclear chromatin stain a dark blue color and are separated by bands staining the same color as the cytoplasm. The cytoplasm stains a very faint sky-blue color and contains a large number of very small reddish-brown granules which in many instances are difficult to observe, so that the cytoplasm has a more or less clear appearance.

Band-formed nuclei are quite common, comprising from two to eight per cent of the total number found. These forms are more common in the younger mink.

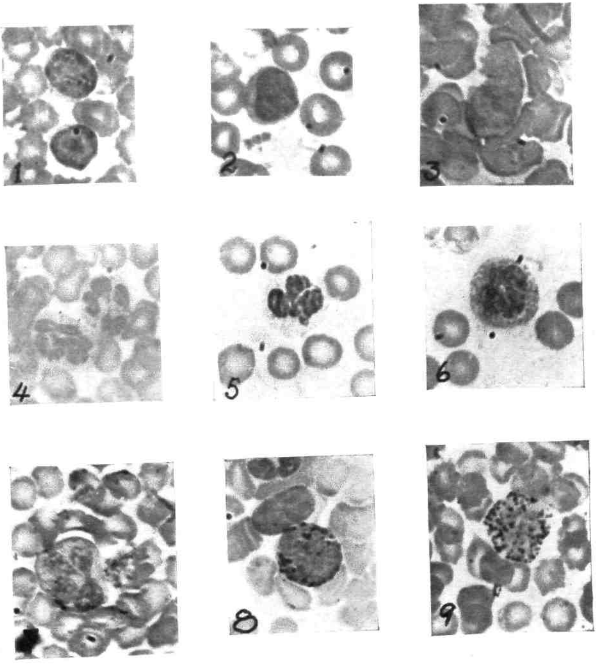
Metamyelocytes were observed and approximate from 0.5 to 2.0% of the total number of neutrophiles.

The average size of the neutrophiles is 14.5 by 12.5 μ. The maximum size is 16.0 by 12.0 μ and the minimum size is 11.5 by 11.0 μ.

Eosinophiles. (Plate I, Figs. 6 and 7). The nuclei of the eosinophile stain lighter blue than those of the neutrophile of mink blood. The lobes are less lobulated and closer together, not being separated by visible chromatin filaments. Since the chromatin masses are not so pronounced, or so prominently divided by lighter staining bands, they give a finer, more granular appearance than the chromatin masses of the neutrophile and basophile.

The cytoplasm stains so lightly that it is almost indistinguishable. Numerous, well defined, dark red to reddish-brown granules, measuring approximately 0.5 μ in diameter, loosely fill the cytoplasm and may obscure portions of the nucleus. The margin of the cell is regular in outline.

The average size is 14.5 by 13.5 μ. The maximum size is 16.0 by 15.0 μ and the minimum 11.5 by 10.0 μ.



FIGS. 1 and 2. *Lymphocytes.* FIG. 3. *Monocyte.*
FIGS. 4 and 5. *Neutrophiles.* FIGS. 6 and 7. *Eosinophiles.*
FIGS. 8 and 9. *Basophiles.* *Mag. 14 X.*

Basophiles. (Plate I, Figs. 8 and 9). The nucleus may be U-shaped or highly lobulated and is then not unlike the neutrophile in form and structure although the lobes are more closely attached to one another. The nucleus stains blue to light purple and is but very little darker than the cytoplasm. The chromatin masses stain more deeply and are separated by narrow pale bands. The cytoplasm stains a pale blue color and has an almost glassy appearance. It may contain from 22 to 65 large prominent granules up to 1 μ in size. The granules appear to stand out from the surface of the cytoplasm and stain dark blue to purple. Granules which are almost indiscernible are also present in the cytoplasm.

The average size of the basophile is 14.0 by 12.0 μ. The maximum is 16.0 by 13.0 μ and the minimum is 11.5 by 11.0 μ.

Degenerated forms of cells found in mink blood. Cells which could not be given a definite classification are placed under this heading. These cells are probably old and degenerate forms which have lost their characteristic identity. It is thought that the majority of them belong to the monocytic and lymphocytic classification. These cells were found more frequently in older mink. The majority of them were irregular in outline, stained deep blue to greenish-blue, and presented a vacuolated and frayed appearance, with no distinguishing characteristics. The so-called basket cells were observed in all classes of mink but appeared more frequently in the adults.

Platelets. Large masses of platelets were often encountered in the blood smears. When massed together they lost their individual identity. When single platelets were observed they were round, oval or irregular in outline. The cytoplasm stains a very light blue and contains fine, deep-blue staining structures, irregular in outline and usually situated in the centre of the cytoplasm in the form of a crescent or circle. Again these structures may be broken up and appear as deposits of fine dust-like particles.

The average size of the platelet is 2.0 by 1.5 μ. The maximum size is 2.5 by 2.0 μ wide and the minimum 1.5 by 1.0 μ.

Acknowledgment

The writer wishes to express his appreciation to Dr. Ronald G. Law, Director of the Ontario Government Experimental Fur Farm, Kirkfield, Ontario, for his interest and assistance throughout the course of the work, and also to thank Mr. Allan Jewell of the same institution for his kind cooperation in the handling of the animals and in the preparation of the manuscript.

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CYTOLOGY OF THE BLOOD OF NORMAL MINK AND RACCOON

II. THE NUMBERS OF THE BLOOD ELEMENTS IN NORMAL MINK[1]

By Arnold H. Kennedy[2]

Abstract

The numbers of lymphocytes, monocytes, neutrophiles, eosinophiles and basophiles in the blood of normal mink of various age groups are listed. Data are given showing the maximum, minimum and average numbers of these types of cells. Similar data are supplied for white and red blood corpuscles and for the amounts of haemoglobin in the blood. Color indices are also given. Basophiles were absent in some males and females of all age groups. Eosinophiles were not distinguished in a number of males and females under two months of age.

In a previous paper (2) the materials and methods used for examining the blood of normal mink were described and a description of the cellular morphology of mink blood was given.

In this paper the numbers of the various types of cells are recorded in Tables I to X. The number of grams of haemoglobin per cubic millimetre (Hb. gm. per cu. mm.) was estimated by the Sahli haemoglobinometer and the haemoglobin index determined by dividing the number of grams of haemoglobin in 100 cc. of blood by the number (in millions) of red blood cells in one cubic millimetre. The formula for calculating the color index is that given by Cullen (1).

The mink are grouped according to age, ranging from under two months of age to adult mink. The red blood corpuscles (R.b.cs.) are estimated to the nearest thousand per cu. mm. of blood and for purposes of tabulation are stated in millions. The total numbers of white blood corpuscles (W.b.cs.) per cu. mm. are recorded without reductions from the actual count. The lymphocyte (Ly.), monocyte (Mo.), neutrophile (Ne.), eosinophile (Eo.) and basophile (Ba.) leucocytes occurring in a cu. mm. of blood are expressed as percentages of the total and also as round numbers.

The numerical range covered by the various groups has been indicated at the foot of each column by tabulating the minimum, maximum and mean figures. The standard deviation has been calculated by dividing the sum of the squares of the deviations from the mean by the number of individual cases recorded, and finding the square root of the result. The standard deviation has been calculated to give all the deviations their true value. In order to have the amounts of variation expressed on a common basis for comparison the coefficient of variation is used. This is the standard deviation expressed as a percentage of the mean.

[1] *Original manuscript received November 30, 1934.*
Contribution from the Ontario Government Experimental Fur Farm, Kirkfield, Ontario.
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Numbers of Blood Elements in Normal Mink

The Red Blood Corpuscles

The average numbers of red blood corpuscles per cubic millimetre of mink blood for all the groups examined were—for males, 7,941,000; for females, 8,133,000. The maximum number for males, 12,288,000, occurred in a mink between five and seven months old. The maximum number for females was 12,360,000 which occurred in a female also between five and seven months of age. The minimum number for all the male groups of mink was 3,704,000 which occurred in a mink kitten 42 days old. The minimum number for all the female groups was 3,584,000 which occurred in a mink kitten 54 days old.

As the age of the mink increased the average number of red blood cells for each group also increased. The greatest differences in individual animals occurred in both males and females between five to seven months old. In these groups the maximum number per cu. mm. of blood for males was 12,288,000 and for females 12,264,000; the minimum number for males was 7,816,000, and for females 7,808,000. Smaller differences in numbers of red blood cells occurred in the adults than in any of the other groups of mink.

TABLE I

NUMBERS OF THE BLOOD ELEMENTS IN MINK UNDER TWO MONTHS OF AGE

Males				Females			
No.	R.b.cs.	Hb. gm.	Ind.	No.	R.b.cs.	Hb. gm.	Ind.
1	5.392	7.9	0.53	1	6.648	8.5	0.40
2	5.328	7.6	0.42	2	6.256	7.7	0.43
3	5.304	8.5	0.57	3	6.872	8.1	0.40
4	5.432	8.2	0.55	4	5.560	7.6	0.42
5	4.808	8.0	0.53	5	4.936	7.8	0.52
6	4.696	8.6	0.57	6	5.536	7.8	0.43
7	6.024	9.0	0.50	7	5.672	8.0	0.44
8	3.704	4.7	0.52	8	5.608	7.5	0.42
9	4.776	5.3	0.44	9	5.792	7.7	0.43
10	5.280	7.7	0.51	10	3.584	6.9	0.57
11	5.880	8.5	0.47	11	5.064	7.2	0.48
12	5.800	8.3	0.46	12	6.160	8.5	0.47
13	6.640	7.0	0.33	—	—	—	—
Min.	3.704	4.7	0.33	Min.	3.584	6.9	0.40
Max.	6.640	9.0	0.57	Max.	6.872	8.5	0.57
Mean	5.324	7.6	0.49	Mean	5.641	7.8	0.45
S.D.	±1.294	±1.2	±0.12	S.D.	±0.819	±0.45	±0.05
C. of V.	26	16	23	C. of V.	15	6	11

Grams of Haemoglobin per 100 cc. of Blood

The average numbers of grams of haemoglobin per 100 cc. of blood for all the groups examined were, for males 10.8, for females 11.8, with a maximum of 17.7 for males and 17.3 for females. These amounts occurred in males

between five and seven months of age and in adult females. The minimum for males (4.7) and for females (6.9), occurred in individuals under two months of age.

TABLE II

NUMBERS OF THE BLOOD ELEMENTS IN MINK UNDER TWO MONTHS OF AGE

Males							Females						
No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
1	6000	74.5	0.0	22.5	2.5	0.5	1	5200	62.5	2.5	34.5	0.5	0.0
		4470	000	1350	150	30			3250	130	1794	26	000
2	6200	44.0	2.0	49.0	4.0	1.0	2	5200	55.5	2.0	42.0	0.0	0.5
		2728	124	3038	248	62			2886	104	2184	000	26
3	8000	64.5	1.0	33.5	1.0	0.0	3	5400	56.0	1.5	38.5	3.0	1.0
		5160	80	2680	80	000			3024	81	2079	162	54
4	8000	67.5	1.0	29.5	1.5	0.5	4	5600	62.5	0.5	35.0	1.5	0.5
		5400	80	2360	120	40			3500	28	1960	84	28
5	4200	74.5	0.0	25.0	0.0	0.5	5	10800	61.5	0.0	38.0	0.0	0.5
		3129	000	1050	000	21			6642	000	4104	000	54
6	7600	66.5	3.5	27.5	1.0	1.5	6	7200	60.5	0.0	38.5	0.5	0.5
		5054	266	2090	76	114			4356	000	2772	36	36
7	7200	71.5	3.0	24.0	0.0	1.5	7	10000	56.5	0.0	43.5	0.0	0.0
		5148	216	1728	000	108			5650	000	4350	000	000
8	9400	62.0	0.5	37.5	0.0	0.0	8	9200	77.5	1.0	19.0	1.5	1.0
		5828	47	3525	000	000			7130	92	1748	138	92
9	8600	63.0	1.0	34.5	0.5	1.0	9	6600	84.5	0.5	15.0	0.0	0.0
		5418	86	2967	43	86			5577	33	990	000	000
10	6600	67.0	1.5	31.5	0.0	0.0	10	7200	78.0	0.0	22.0	0.0	0.0
		4422	99	2079	000	000			5616	000	1584	000	000
11	5800	70.0	2.0	27.5	0.0	0.5	11	7600	62.5	2.0	35.0	0.5	0.0
		4060	116	1595	000	29			4750	152	2660	38	000
12	6600	59.0	0.5	39.5	1.0	0.0	12	9400	88.0	0.5	11.0	0.5	0.0
		3894	33	2607	66	000			8272	47	1034	47	000
13	6600	72.0	1.5	26.0	0.5	0.0	—	—	—	—	—	—	—
		4752	99	1716	33	000			—	—	—	—	—
Min.	4200	44.0	0.0	22.5	0.0	0.0	Min.	5200	55.5	0.0	11.0	0.0	0.0
Max.	9400	74.5	3.5	49.0	4.0	1.5	Max.	10800	88.0	2.5	42.0	3.0	1.0
Mean	7000	65.8	1.3	31.3	0.9	0.5	Mean	7450	67.1	0.9	31.0	0.7	0.3
S.D.	±1304	±7.7	±1.0	±7.1	±1.1	±0.5	S.D.	±1898	±11.09	±0.86	±10.6	±0.89	±0.37
C. of V.	19	12	77	23	122	100	C. of V.	25	17	96	34	127	123
Min.		2728	000	1050	000	000	Min.		2886	000	990	000	000
Max.		5828	266	3525	248	114	Max.		7130	152	4350	162	92
Mean		4574	96	2214	63	38	Mean		5054	56	2272	44	24
S.D.		±884	±73	±699	±72	±40	S.D.		±1658	±51	±1015	±52	±29
C. of V.		19	76	31	114	105	C. of V.		33	91	45	118	121

The amounts of haemoglobin increased with age in the female groups which show an average of 15.0 gm. in the adult group. In the male groups the amounts increased up to seven months of age when the average for the group was 14.3 gm. In the adult males there was a decrease, the average for the group being 11.9 gm.

Color Index

For the color index, the haemoglobin content of the red blood cells was calculated as gm. haemoglobin in 100 cc. of blood per one million red blood cells in one cubic millimetre.

The averages for all groups examined were .46 for males and .48 for females. Extreme limits for males were .76 to .33; for females .64 to .39. The highest index reached in male mink occurred between two and four months of age, and in female mink between five and seven months of age. The lowest index reached in male mink was in an individual under two months old and the lowest in female mink in an animal between two and four months old.

The female adult mink showed a higher color index than the adult males. The averages were:—adult female group .52, adult male group .41.

TABLE III

NUMBERS OF THE BLOOD ELEMENTS IN MINK OVER TWO AND UNDER FIVE MONTHS OF AGE

Males				Females			
No.	R.b.cs.	Hb. gm.	Ind.	No.	R.b.cs.	Hb. gm.	Ind.
1	7.840	10.2	0.42	1	7.992	11.5	0.48
2	7.208	9.4	0.45	2	9.200	10.5	0.39
3	7.712	10.6	0.44	3	8.744	10.4	0.38
4	4.200	9.1	0.76	4	8.464	10.2	0.42
5	6.560	9.8	0.47	5	7.784	9.9	0.41
6	6.896	9.4	0.45	6	7.752	9.3	0.39
7	7.472	10.5	0.50	7	7.528	9.9	0.41
8	6.176	9.5	0.53	8	6.752	9.9	0.47
9	7.768	8.9	0.37	9	5.536	9.7	0.54
10	8.008	9.5	0.40	10	7.360	9.9	0.47
11	8.552	9.5	0.35	11	5.432	8.9	0.59
12	8.112	9.0	0.37	12	7.912	10.5	0.44
13	6.720	9.5	0.45	13	7.096	9.5	0.45
Min.	4.200	8.9	0.35	Min.	5.432	8.9	0.38
Max.	8.552	10.6	0.76	Max.	9.200	11.5	0.59
Mean	7.171	9.6	0.46	Mean	7.489	10.0	0.45
S.D.	±1.051	±0.51	±0.01	S.D.	±0.967	±0.6	±0.06
C. of V.	15	5	2	C. of V.	13	6	13

White Blood Corpuscles

The average number of white blood corpuscles for all male groups examined was 7,902. A maximum of 16,400 occurred in a mink between five and seven months of age and a minimum of 3,800 in an adult mink. The average

number of white blood cells found in the female mink was 8,047. A maximum of 15,000 occurred in a mink between five and seven months of age. A minimum of 4,600 occurred in a mink between five and seven months of age.

TABLE IV

NUMBERS OF THE BLOOD ELEMENTS IN MINK OVER TWO AND UNDER FIVE MONTHS OF AGE

Males							Females						
No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
1	8200	48.0	0.5	47.5	4.0	0.0	1	8200	55.0	0.0	29.5	15.5	0.0
		3936	41	3895	328	000			4510	000	2419	1271	000
2	15400	54.5	0.5	28.0	16.5	0.5	2	10400	73.0	0.5	19.0	7.0	0.5
		8393	77	4312	2541	77			7592	52	1976	728	52
3	7400	44.5	0.5	54.0	1.0	0.0	3	7600	50.0	2.0	36.5	11.0	0.5
		3293	37	3996	74	000			3800	152	2774	836	38
4	11200	33.0	1.0	64.5	1.0	0.5	4	10600	59.5	1.0	35.0	4.0	0.5
		3696	112	7224	112	56			6307	106	3710	424	53
5	12400	25.0	1.0	70.0	2.0	2.0	5	6800	57.5	0.0	39.0	3.5	0.0
		3100	124	8680	248	248			3910	000	2652	238	000
6	6800	31.0	5.0	53.0	9.5	1.5	6	12200	55.5	0.0	40.0	4.5	0.0
		2108	340	3604	646	102			6771	000	4880	549	000
7	8400	33.5	1.5	60.0	3.5	1.5	7	7600	71.0	1.0	25.5	1.5	0.5
		2814	126	5040	294	126			5434	76	1938	114	38
8	6800	54.0	0.5	39.0	6.0	0.5	8	11000	49.5	1.5	46.5	1.5	1.0
		3672	34	2652	408	34			5445	165	5115	165	110
9	7200	37.5	2.5	58.5	1.5	0.0	9	12800	43.5	0.0	45.5	10.5	0.5
		2700	180	4212	108	000			5568	000	5824	1344	64
10	6800	32.0	3.0	52.0	10.0	3.0	10	8600	29.5	1.5	67.0	2.0	0.0
		2176	204	3536	680	204			2537	129	5762	172	000
11	5000	49.0	1.0	41.0	7.0	2.0	11	9800	51.5	2.5	44.5	1.0	0.5
		2450	50	2050	350	100			5047	245	4361	98	49
12	10400	25.0	3.0	70.5	1.0	0.5	12	5600	36.5	4.0	51.0	7.0	1.5
		2600	312	7332	104	52			2044	224	2856	392	84
13	9200	41.0	3.0	47.5	8.5	0.0	13	5800	37.5	2.0	53.5	7.0	0.0
		3772	276	4370	782	000			2175	116	3103	406	000
Min.	5000	25.0	0.5	28.0	1.0	0.0	Min.	5600	29.5	0.0	19.0	1.0	0.0
Max.	15400	54.5	5.0	70.5	16.5	3.0	Max.	12800	73.0	4.0	67.0	15.5	1.5
Mean	8862	39.0	1.8	52.7	5.5	0.9	Mean	9000	52.0	1.2	41.0	5.8	0.4
S.D.	±2719	±9.8	±1.3	±11.8	±4.5	±0.9	S.D.	±2285	±12.3	±1.1	±12.1	±4.2	±0.4
C. of V.	31	25	72	22	82	100		25	24	92	29	72	100
Min.		2108	34	2050	74	000	Min.		2044	000	1938	98	000
Max.		8393	340	8680	2541	248	Max.		7592	245	5824	1271	110
Mean		3439	147	4685	513	77	Mean		4703	97	3644	518	38
S.D.		±1548	±32	±1853	±627	±76	S.D.		±1592	±82	±1339	±401	±35
C. of V.		45	22	40	122	99	C. of V.		34	85	37	77	92

The average number of white blood cells showed a steady increase in the young mink, with greater differences between individual animals as the groups increased in age. The white blood cells decreased in numbers in adult mink, the average number for the males being 6,380 and for females 7,800.

Lymphocytes

For all male groups the lymphocytes averaged 3,957 (49.0%) cells per cu. mm. of blood and for the female groups the average was 4,269 (53.1%). The maximum number of lymphocytes in the male groups was 11,562 (70.5%) found in a mink between five and seven months old, and the minimum was 900 (22.5%). The latter occurred in the adult male group. The maximum number for the female groups was 10,875 (72.5%) found in a mink between five and seven months old and the minimum was 1,470 (21.0%) also in a mink between five and seven months old. The greatest differences occurred in the groups between five and seven months of age. Greater differences occurred among female mink than among males. Male and female mink under two months of age showed a higher proportion of lymphocytes in the blood than those of any of the other age groups. The maximum proportion of lymphocytes in males, 74.5%, occurred in two individuals; the minimum proportion was 44.0% and the average proportion for the group 65.8%. The maximum proportion of lymphocytes in female mink of the same age was 88.0%; the minimum proportion was 55.5% and the average proportion for the group 67.1%.

TABLE V

NUMBERS OF THE BLOOD ELEMENTS IN MINK OVER FIVE AND UNDER SEVEN MONTHS OF AGE

Males				Females			
No.	R.b.cs.	Hb. gm.	Ind.	No.	R.b.cs.	Hb. gm.	Ind.
1	7.816	10.0	0.41	1	7.808	11.3	0.47
2	8.832	11.5	0.42	2	9.952	13.0	0.43
3	8.976	13.3	0.49	3	8.752	13.0	0.48
4	8.968	13.5	0.50	4	7.880	12.8	0.53
5	9.584	15.4	0.51	5	9.472	17.2	0.64
6	11.056	17.7	0.54	6	9.912	15.4	0.51
7	12.288	16.7	0.46	7	12.176	15.0	0.42
8	9.928	16.7	0.56	8	12.264	15.8	0.44
9	9.072	16.0	0.59	9	9.288	14.9	0.55
10	8.960	12.4	0.46	10	9.288	16.0	0.59
Min.	7.816	10.0	0.41	Min.	7.808	11.3	0.42
Max.	12.288	17.7	0.59	Max.	12.264	17.2	0.64
Mean	9.548	14.3	0.49	Mean	9.679	14.4	0.51
S.D.	±1.212	±2.42	±0.06	S.D.	±1.344	±1.7	±0.06
C. of V.	13	17	12	C. of V.	14	12	12

Monocytes

The average number of monocytes occurring in the blood of male mink for all groups examined was 157 (1.8%) with extreme limits of 627 (5.5%)

to 0, the last in a mink between five and seven months of age. The average for females was 90 (1.2%) with limits of 420 (5.0%) to 0 occurring in the adults. The greatest number of monocytes was found in mink between five and seven months of age. The smallest number of monocytes was found in mink under two months of age. The greatest fluctuations among the individual animals occurred in the adult groups. The smallest fluctuations were found in the mink under two months of age.

TABLE VI

NUMBERS OF THE BLOOD ELEMENTS IN MINK OVER FIVE AND UNDER SEVEN MONTHS OF AGE

Males							Females						
No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
1	8000	62.5	2.5	28.0	7.0	0.0	1	13200	32.0	1.0	65.0	2.0	0.0
		5000	200	2240	560	000			4224	132	8580	264	000
2	11400	49.0	5.5	30.5	14.5	0.5	2	6000	36.5	1.5	52.5	9.0	0.5
		5586	627	3477	1653	57			2190	90	3150	540	30
3	8000	43.0	3.5	44.5	8.0	1.0	3	9600	66.0	3.0	28.5	2.0	0.5
		3440	280	3560	640	80			6336	288	2736	192	48
4	10000	53.5	1.5	40.0	4.5	0.5	4	15000	72.5	1.0	19.0	7.5	0.0
		5350	150	4000	450	50			10875	150	2850	1125	000
5	13200	60.5	3.0	29.0	7.5	0.0	5	6000	49.5	1.0	36.0	13.5	0.0
		7986	396	3828	990	000			2970	60	2160	810	000
6	16400	70.5	2.0	19.0	8.5	0.0	6	6400	27.0	2.5	64.0	6.5	0.0
		11562	328	3116	1394	000			1728	160	4096	416	000
7	10600	57.5	2.5	36.5	3.0	0.5	7	5400	54.5	0.5	39.5	4.0	1.5
		6095	265	3869	318	53			2943	27	2133	216	81
8	5800	29.5	5.0	57.5	8.0	0.0	8	7000	21.0	0.0	74.5	4.5	0.0
		1711	290	3335	464	0.0			1470	000	5215	315	000
9	5400	31.5	3.5	54.5	10.0	0.5	9	6200	35.0	0.0	65.0	0.0	0.0
		1701	189	2943	540	27			2170	000	4030	000	000
10	5000	19.5	0.0	76.5	4.0	0.0	10	4600	63.0	0.5	32.5	4.0	0.0
		975	000	3825	200	000			2898	23	1495	184	000
Min.	5000	19.5	0.0	19.0	3.0	0.0	Min.	4600	21.0	0.0	19.0	0.0	0.0
Max.	16400	70.5	5.5	76.5	14.5	1.0	Max.	15000	72.5	3.0	74.5	13.5	1.5
Mean	9380	47.70	2.90	41.60	7.50	.30	Mean	7940	45.70	1.10	47.70	5.30	.25
S.D.	±3485	±15.64	±1.53	±12.99	±3.14	±0.40	S.D.	±3189	±16.9	±0.81	±17.7	±3.8	±0.45
C. of V.	37	33	53	31	42	133	C. of V.	40	37	74	37	72	140
Min.		975	150	2240	200	000	Min.		1470	000	1495	000	000
Max.		11562	627	4000	1653	80	Max.		10875	288	8580	1125	81
Mean		4941	273	3419	721	27	Mean		3780	93	3645	406	16
S.D.		±3045	±164	±510	±440	±29	S.D.		±2718	±87	±1949	±320	±27
C. of V.		62	60	15	61	108	C. of V.		72	94	53	79	169

Neutrophiles

The average number of neutrophiles for the male mink groups examined was 3,299 (42.3%); and that for the female groups was 3,311 (41.5%). The extreme limits for the males were 8,680 (70.0%) and 1,050 (25.0%). Limits for the females were 8,580 (65.0%) and 1,034 (11.0%). The lowest proportion of neutrophiles for the individual animals was found in the mink under two months of age. There is a slight increase in the average numbers of

TABLE VII

NUMBERS OF THE BLOOD ELEMENTS IN ADULT MINK

Males				Females			
No.	*R.b.cs.*	*Hb. gm.*	*Ind.*	*No.*	*R.b.cs.*	*Hb. gm.*	*Ind.*
1	9.512	10.2	0.34	1	8.336	11.7	0.49
2	9.176	9.9	0.37	2	7.504	11.4	0.48
3	9.800	9.9	0.33	3	8.624	16.0	0.59
4	10.352	10.7	0.36	4	11.320	17.0	0.51
5	9.872	9.5	0.32	5	9.104	16.0	0.59
6	8.952	12.5	0.46	6	11.040	17.3	0.52
7	9.016	12.9	0.48	7	10.784	15.1	0.46
8	9.552	13.6	0.45	8	10.248	16.1	0.54
9	10.424	14.4	0.48	9	9.544	14.5	0.48
10	10.176	15.6	0.52	—	—	—	—
Min.	8.952	9.5	0.32	Min.	7.504	11.4	0.46
Max.	10.424	15.6	0.52	Max.	11.320	17.3	0.59
Mean	9.683	11.9	0.41	Mean	9.723	15.0	0.52
S.D.	±0.507	±2.06	±0.07	S.D.	±1.254	±2.0	±0.04
C. of V.	5	17	17	C. of V.	13	13	8

neutrophiles per cu. mm. of blood from the young female to the adult female mink. In the group under two months of age the number was 2,272 (31.0%). In adults the number was 3,683 (46.3%). The highest average number for the male groups was 4,685 (52.7%), found in males between two and four months of age. The lowest number, 2,214 (31.3%), was found in the male group under two months of age. In the adult male group the average number decreased to 2,880 (47.1%).

Eosinophiles

The average number of eosinophiles per cu. mm. of blood for all groups examined was: males 438 (5.3%), with extreme limits of 2,541 (16.5%) to 0; females 314 (3.9%), with extreme limits of 1,344 (10.5%) to 0. The highest average number of eosinophiles for all the male groups was in the group five to seven months of age, where 721 (7.5%) per cu. mm. of blood was found. The lowest average number for the male groups, 63 (0.9%), was found in the group under two months of age. The highest average number for the female groups was found in the group two to four months of age which showed 518 (5.8%) per cu. mm. of blood. The lowest average number

for all the female groups, 44 (0.7%), was found in the group under two months of age. In a number of the animals in both male and female groups of mink under two months of age the presence of eosinophiles was not established.

Basophiles

The average number of basophiles occurring in the blood of male mink for all the groups examined was 49 (0.6%), with extreme limits of 248 (2.0%)

TABLE VIII

NUMBERS OF THE BLOOD ELEMENTS IN ADULT MINK

Males							Females						
No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
1	7400	46.0	1.5	45.0	6.5	1.0	1	8600	50.5	4.5	41.0	3.0	1.0
		3404	111	3330	481	74			4343	387	3526	258	86
2	10200	47.5	4.0	42.5	5.5	0.5	2	8400	30.0	5.0	51.0	13.0	1.0
		4845	408	4335	561	51			2520	420	4284	1092	84
3	4800	47.0	1.0	48.5	2.5	1.0	3	8800	39.5	0.5	58.5	0.0	1.5
		2256	48	2328	120	48			3476	44	5148	000	132
4	5000	50.5	1.0	42.0	5.5	1.0	4	7000	68.5	0.0	26.5	4.0	1.0
		2525	50	2100	275	50			4795	000	1855	280	70
5	9600	46.0	0.0	50.0	3.0	1.0	5	6200	67.0	0.0	29.0	4.0	0.0
		4416	000	4800	288	96			4154	000	1798	248	000
6	6000	57.5	1.5	25.0	15.5	0.5	6	7200	44.0	0.5	51.0	3.0	1.5
		3450	90	1500	930	30			3168	36	3672	216	108
7	7200	57.5	5.5	18.5	16.0	2.5	7	12200	33.5	0.5	65.5	0.0	0.5
		4140	396	1332	1152	180			3752	56	7336	000	56
8	3800	33.5	0.0	63.5	3.0	0.0	8	6600	55.0	0.0	44.0	0.5	0.5
		1273	000	2413	114	000			3630	000	2904	33	33
9	5800	26.5	0.0	69.0	4.5	0.0	9	5200	39.0	2.0	50.5	8.5	0.0
		1537	000	4002	261	000			2028	104	2626	442	000
10	4000	22.5	1.0	66.5	9.5	0.5	—	—	—	—	—	—	—
		900	40	2660	380	20			—	—	—	—	—
Min.	3800	22.5	0.0	18.5	2.5	0.0	Min.	5200	30.0	0.0	26.5	0.0	0.0
Max.	10200	57.5	5.5	69.0	16.0	2.5	Max.	12200	68.5	5.0	65.5	13.0	1.5
Mean	6380	43.5	1.1	47.1	7.2	0.8	Mean	7800	47.5	1.5	46.3	4.0	0.8
S.D.	±2095	±11.5	±1.8	±15.8	±4.7	±0.68	S.D.	±1496	±12.1	±1.9	±12.0	±4.0	±0.54
C. of V.	33	26	164	34	65	85	C. of V.	19	25	127	26	100	66
Min.		900	000	1332	114	000	Min.		2028	000	1798	000	000
Max.		4845	408	4800	1152	180	Max.		4795	420	7336	1092	132
Mean		2875	114	2880	456	55	Mean		3541	116	3683	285	63
S.D.		±1312	±149	±1129	±325	±51	S.D.		±831	±157	±1675	±318	±42
C. of V.		46	130	39	71	93	C. of V.		23	135	45	112	67

to 0 occurring in mink between two and five months of age. The average for females was 35 (0.4%) with limits of 132 (1.5%) to 0 occurring in adult mink. The highest average number of basophiles for the male group, 77 (0.9%) was found in the group from two to five months of age. Among the females the highest average number, 63 (0.8%), was found in the adult

TABLE IX

FINAL MEANS OF THE BLOOD ELEMENTS IN ALL GROUPS OF MINK

Males				Females			
Age, months	R.b.cs.	Hb. gm.	Ind.	Age, months	R.b.cs.	Hb. gm.	Ind.
0–2	5.324	7.6	0.49	0–2	5.641	7.8	0.45
2–3	7.171	9.6	0.46	2–3	7.489	10.0	0.45
5–7	9.548	14.3	0.49	5–7	9.679	14.4	0.51
Yr. 1–3	9.683	11.9	0.41	Yr. 1–3	9.723	15.0	0.52
Min.	5.324	7.6	0.41	Min.	5.641	7.8	0.45
Max.	9.683	14.3	0.49	Max.	9.723	15.0	0.52
Mean	7.941	10.8	0.46	Mean	8.133	11.8	0.48
S.D.	±1.723	±3.0	±0.03	S.D.	±1.918	±2.5	±0.04
C. of V.	22	27	6	C. of V.	24	21	8

TABLE X

FINAL MEANS OF THE BLOOD ELEMENTS IN ALL GROUPS OF MINK

Males							Females						
Age, months	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	Age, months	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
0–2	6985	65.8	1.3	31.3	0.9	0.5	0–2	7450	67.1	0.9	31.0	0.7	0.3
		4574	96	2214	63	38			5054	56	2272	44	24
2–3	8862	39.0	1.8	52.7	5.5	0.9	2–3	9000	52.0	1.2	41.0	5.8	0.4
		3439	147	4685	513	77			4703	97	3644	518	38
5–7	9380	47.7	2.9	41.6	7.5	0.3	5–7	7940	45.7	1.1	47.7	5.3	0.2
		4941	273	3419	721	27			3780	93	3645	406	16
Yr.	6380	43.5	1.1	47.1	7.2	0.8	Yr.	7800	47.5	1.5	46.3	4.0	0.8
1–3		2875	114	2880	456	55	1–3		3541	116	3683	285	63
Min.	6380	39.0	1.1	31.3	0.9	0.3	Min.	7450	45.7	0.9	31.0	0.7	0.2
Max.	9380	65.8	2.9	52.7	7.5	0.9	Max.	9000	67.1	1.5	47.7	5.8	0.8
Mean	7902	49.0	1.8	43.2	5.3	0.6	Mean	8047	53.1	1.2	41.5	3.9	0.4
S.D.	±578	±8.4	±0.7	±6.0	±2.6	±0.2	S.D.	±1251	±10.2	±0.7	±7.9	±2.6	±0.2
C. of V.	7	17	39	14	49	33	C. of V.	16	19	58	19	67	50
Min.		2875	96	2214	63	38	Min.		3541	56	2272	44	16
Max.		4941	273	4685	721	77	Max.		5054	116	3683	518	63
Mean		3957	157	3299	438	49	Mean		4269	90	3311	314	35
S.D.		±627	±22	±600	±176	±18	S.D.		±835	±69	±907	±238	±18
C. of V.		16	14	18	40	37	C. of V.		20	77	27	76	51

group. The lowest average number for all the male groups, 38 (0.5%), was found in the group under two months of age. The lowest average number for the female groups, 16 (0.2%) was found in the group between five and seven months of age. In all the groups of both the male and female mink, a number of individual animals did not reveal the presence of basophiles in their blood.

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CYTOLOGY OF THE BLOOD OF NORMAL MINK AND RACCOON

III. MORPHOLOGY AND NUMBERS OF THE BLOOD ELEMENTS IN RACCOON[1]

By Arnold H. Kennedy[2]

Abstract

Maximum, minimum and average values for numbers and dimensions of the elements in the blood of normal raccoon are given. Normoblasts are rare and anisocytosis was found only in animals from one to three months old. Lymphocytes could not be classified into size groups as they completely cover a wide range from small to large. In the monocytes the nuclei are prominent and boldly outlined but vary in shape. In the neutrophiles the nuclei have from two to seven segments, five being most common. The eosinophiles are large and contain oval, S-shaped, or lobed nuclei. The nuclei of the basophiles are usually bar- or S-shaped, seldom lobed. Two types of basophilic cells may be distinguished. One contains 8–40 prominent granules while the other contains a mass of granular material with occasionally a few large granules. In addition to descriptions of these elements, data are supplied on haemoglobin content and color index of raccoon blood.

The methods used for examining the raccoon blood were those described in previous papers (1, 2).

Blood examinations made on raccoon during the hibernating season are not being considered at this time. The raccoon were grouped according to age, ranging from under three months to adults. The cellular morphology and the numbers of the blood elements for raccoon are considered here.

Morphology of Raccoon Blood

The red blood corpuscles take up the eosin from Hasting's stain, giving a slight orange red to dark red color. The staining is diffused evenly throughout the cell and no fine structures are visible. The centre appears paler than the periphery, owing to the biconcave formation of the corpuscle.

The average diameter of the red blood corpuscle is 6.5 μ with extreme limits of 5.0 to 9.0 μ.

Normoblasts were very rare in raccoon blood, only one or two being observed in the examination of all the films from the various age groups.

Anisocytosis was not found to any extent except in raccoon from one to three months old.

Lymphocytes (Plate I, Figs. 1 and 2)

The lymphocytes of the raccoon appear very much like the lymphocytes of the mink (1). Two forms are found, one large, the other small. Intermediate forms occur, making it impossible to separate the lymphocytes into two distinct groups. The small forms are by far the most numerous. In the larger forms the nucleus may be rounded, bean-shaped or irregular in outline. It may be situated near the margin of the cell or more centrally located, and stains a light purple color with the nuclear material appearing

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Contribution from the Ontario Government Experimental Fur Farm, Kirkfield, Ontario.
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more uniform and granular in appearance than in the smaller forms. The nuclei of the larger lymphocytes are surrounded by a greater amount of pale blue staining cytoplasm than are the nuclei of the smaller forms.

The small lymphocytes have a small, compact, round or oval nucleus, with the nuclear masses staining a deep purple color. The heavier staining nuclear masses are separated by clear open spaces which give the nuclei an uneven, slightly wavy appearance. The nucleus may be surrounded by a light-blue staining cytoplasm, which in some cells can hardly be discerned owing to the light staining, or by a very narrow margin of cytoplasm, but a narrow ring or crescent on one side is the most common form.

The lymphocytic cytoplasm may contain from three to sixteen reddish-brown granules. These may easily be discerned whenever they occur.

The largest lymphocyte observed was 19.0 by 12.0 μ, with the nuclei 12.0 by 12.0 μ. The average size was 11.0 by 9.4 μ with the nuclei 10.7 by 8.2 μ. The minimum size was 6.0 by 5.0 μ, with the nuclei 5.0 by 5.0 μ.

Monocytes (Plate I, Figs. 3 and 4)

The cytoplasm of the monocytes stains a light blue or faint greenish-blue color. No granules were observed. The nuclei are prominent, of various shapes and boldy outlined. Kidney-, band-, horseshoe-, and L-shaped forms were most common. In the smaller monocytes the nucleus appeared more rounded, thicker, and oval. Occasionally a nucleus with three lobes would be observed. These occurred more often in the larger monocytes. The nucleus, staining from a light to a reddish purple, is usually located near the outer margin of the cell with the convex side towards the margin. The more rounded nuclei are often more centrally located. The maximum size of the monocyte is 18.0 by 12.0 μ and the average size 14.8 by 10.8 μ; the size of the forms most commonly found lies between 12.0 and 14.0 μ. The minimum size is 10.3 by 9.0 μ.

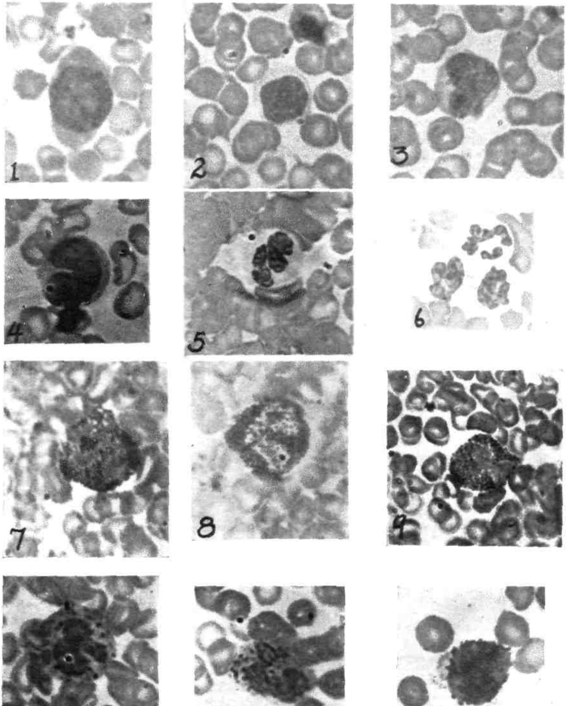
Neutrophiles (Plate I, Figs. 5 and 6)

The cytoplasm of the neutrophile stains a very light, sky-blue color. A number of small, faintly stained, reddish-blue granules may be discerned throughout the cytoplasm, especially in young raccoons' blood. The granules stain so faintly in many cells that their presence may often be unnoticed. The nuclei stain from deep blue to purple, are segmented and sharply divided into bands by the oxyphile portion of the nuclear material. The numbers of segments vary from two to seven, five being the most common. The segments are connected by fine, very distinct filaments of chromatin. Band-form nuclei are observed, especially in the younger groups of raccoon, and constitute from two to seven per cent of the total number of neutrophiles.

Metamyelocytes are also observed and approximate from 0.5 to 2.0% of the total number of neutrophiles. Occasionally a myelocyte may be observed but they appear to be very rare in raccoon blood.

The maximum size of the neutrophiles is 16.0 by 13.0 μ; the average size is 12.8 by 11.0 μ and the minimum size is 10.2 by 8.2 μ.

PLATE I



FIGS. 1 and 2. *Lymphocytes.* FIGS. 3 and 4. *Monocytes.* FIGS. 5 and 6. *Neutrophiles.* FIGS. 7, 8 and 9. *Eosinophiles.* FIGS. 10, 11 and 12. *Basophiles.* × 14.

Eosinophiles (Plate I, Figs. 7, 8 and 9)

The eosinophile in raccoon blood is large, prominent and bold in outline, with a tendency to have slightly frayed or roughened borders. The granules stain bright red to pink. They are numerous, large, and prominent, and fill up the cell, often projecting over the borders, and they may completely obscure the cytoplasm and portions of the nucleus. The nucleus is very simple, frequently oval, S-shaped, or in the form of a curved bar or divided into two or three lobes, which in all probability are joined by chromatin threads, but the threads are always obscured by the granules. The nuclei stain from a sky-blue to a deep blue or purplish color. The chromatin usually has a uniform appearance. In broken down cells the granules can be seen more clearly. They appear round to oval in shape and measure approximately 0.5 to 0.7 μ.

The maximum size of the eosinophile is 18.0 by 13.0 μ; the average size is 13.0 by 10.4 μ and the minimum 11.0 by 6.0 μ.

Basophiles (Plate I, Figs. 10, 11 and 12)

The nuclei of the basophile cells are usually quite prominent and not obscured to any extent by granules. They are usually band-, bar-, or S-shaped and are very seldom divided or lobed. They stain a light purple color and are divided into bands by the oxyphile portion of the nuclear material, which takes a color similar to that taken by the cytoplasm of the cell. In staining reaction and appearance, they are very similar to band-formed neutrophiles. The basophiles, in raccoon blood, contain two types of granules; these types occurring in different cells. The greater number of basophile cells contain numerous granules which often fill the cell. These stain a very faint light-blue to a faint smoky-blue color. They are about the size of the granules of the eosinophile cells or a little smaller, but are less prominently defined in outline. Because of their faint, indistinct appearance these smaller granules may often be overlooked, and the cell classified as a neutrophile. A few large deep-purple granules may be present in the cytoplasm of these cells. Basophilic cells are also found which contain not the smaller, more faintly staining granules which fill the cytoplasm, but prominent large sized granules, 8 to 40 in number, measuring around 1.0 μ and staining a bright purple color. These granules may appear over the nucleus and they also appear on the outer surface of the cytoplasm (Plate I, Fig. 12). The cytoplasm of these cells stains lilac or light mauve. The number of basophiles in the raccoon is too low to give any accurate indication of the proportions of the two types of cells.

Both types of basophilic cells are of approximately the same size. The maximum size is 15.0 by 11.0 μ, the average 13.2 by 10.5 μ and the minimum 11.0 by 9.0 μ.

Degenerate Forms of Cells Found in Raccoon Blood

Under this heading are placed those cells that have lost their characteristic identity and possess no definite structure. The majority of them most

probably are lymphocytic and monocytic in origin. They stain greenish blue to dark blue and often have a faded and frayed appearance.

Platelets

Platelets often appeared in large masses and their individual identity could not be discerned. Where they appeared singly they were round, oblong or irregular in outline. The protoplasm stained a pale, sky-blue color and contained clumps or rings of dark-blue-staining structures throughout the cytoplasm.

The maximum size for platelets in raccoon blood is 5.0 by 3.0 μ. The average size is 3.0 by 2.5 μ, and the minimum 2.0 by 2.0 μ.

Numbers of Blood Elements in Normal Raccoon

The numbers of the various types of cells in raccoon blood are recorded in Tables I–X. The raccoon are grouped according to age, ranging from under three months to adults one year and over. The same technique and system were used in expressing the red blood corpuscles, haemoglobin content, color index, total white blood corpuscles, lymphocyte, monocyte, neutrophile, eosinophile and basophile leucocytes, the minimum, maximum and mean figures, the standard of deviation and coefficient of variation for raccoon blood, as were used in a previous paper (2).

TABLE I

Numbers of the blood elements in raccoon under three months of age

Males				Females			
No.	R.b.cs.	Hb. gm.	Ind.	No.	R.b.cs.	Hb. gm.	Ind.
1	7.752	5.5	0.23	1	8.280	5.0	0.21
2	8.008	5.5	0.23	2	8.280	4.9	0.20
3	9.296	6.7	0.25	3	6.296	4.8	0.26
4	9.528	6.0	0.20	4	7.136	5.2	0.25
5	8.672	6.3	0.23	5	7.968	5.3	0.22
6	8.896	6.6	0.24	6	8.288	5.5	0.23
7	7.944	6.0	0.25	7	7.856	5.8	0.24
8	6.976	5.1	0.24	8	8.376	5.9	0.25
—	—	—	—	9	7.928	6.0	0.25
—	—	—	—	10	8.424	6.5	0.27
—	—	—	—	11	8.352	5.6	0.23
—	—	—	—	12	8.752	5.6	0.21
Min.	6.976	5.1	0.20	Min.	6.296	4.8	0.21
Max.	9.528	6.7	0.25	Max.	8.752	6.5	0.27
Mean	8.384	5.96	0.23	Mean	7.995	5.5	0.23
S.D.	±0.806	±0.6	±0.04	S.D.	±0.640	±0.5	±0.06
C. of V.	10	10	17	C. of V.	8	9	26

Red Blood Corpuscles

The average number of red blood corpuscles per cubic millimetre of raccoon blood in all the groups examined was, males 10,324,000; females 9,749,000.

A maximum number of 13,368,000 for males occurred in a raccoon between seven and twelve months of age. The maximum number for the females, 13,592,000, occurred in an adult. The minimum number in the male groups, 6,976,000, was found in a raccoon under three months of age. The maximum number in all the female groups, 6,296,000, was also found in a raccoon under three months old.

TABLE II

NUMBERS OF THE BLOOD ELEMENTS IN RACCOON UNDER THREE MONTHS OF AGE

Males							Females						
No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
1	16400	55.0 9020	0.5 82	40.0 6560	4.5 738	0.0 000	1	12400	56.5 7006	0.5 62	40.5 5022	2.5 310	0.0 000
2	10400	50.5 5252	1.0 104	44.0 4576	4.5 468	0.0 000	2	19000	— —	— —	— —	— —	— —
3	6800	55.0 3740	0.0 000	44.0 2992	1.0 68	0.0 000	3	10600	72.5 7685	0.0 000	25.0 2650	2.5 265	0.0 000
4	9000	65.6 5895	2.0 180	31.5 2835	1.0 90	0.0 000	4	14600	51.0 7446	0.0 000	46.0 6716	3.0 438	0.0 000
5	7400	53.0 3922	2.5 185	43.5 3219	1.0 74	0.0 000	5	14000	66.0 9240	1.0 140	31.0 4340	1.5 210	0.5 70
6	9600	54.0 5184	1.0 96	44.0 4224	1.0 96	0.0 000	6	11800	48.0 5664	0.0 000	46.0 5428	6.0 708	0.0 000
7	8600	48.0 4128	0.0 000	51.0 4386	1.0 86	0.0 000	7	14600	53.0 7738	0.0 000	43.5 6351	3.5 511	0.0 000
8	8000	45.5 3640	0.0 000	53.0 4240	1.5 120	0.0 000	8	17400	53.0 9222	0.5 87	36.0 6264	10.5 1827	0.0 000
—	—	— —	— —	— —	— —	— —	9	6600	60.0 3960	0.5 33	37.0 2475	2.0 132	0.5 33
—	—	— —	— —	— —	— —	— —	10	11800	60.0 7080	1.0 118	38.0 4484	0.5 59	0.5 59
—	—	— —	— —	— —	— —	— —	11	13200	67.0 8844	0.0 000	30.5 4026	2.5 330	0.0 000
—	—	— —	— —	— —	— —	— —	12	8200	49.5 4059	0.5 41	47.0 3854	3.0 246	0.0 000
Min.	6800	45.5	0.0	31.5	1.0	0.0	Min.	6600	48.0	0.0	25.0	0.5	0.0
Max.	16400	65.5	2.5	53.0	4.5	0.0	Max.	19000	72.5	1.0	47.0	10.5	0.5
Mean	9525	53.5	0.9	44.0	1.9	0.0	Mean	12850	58.0	0.4	38.2	3.4	0.13
S.D.	±2818	±5.6	±0.9	±6.1	±1.5	±0.0	S.D.	±5392	±7.6	±0.6	±6.9	±2.6	±0.49
C. of V.	30	10	100	14	79	000	C. of V.	42	13	100	2	8	377
Min.		3640	000	2835	68	000	Min.		3960	000	2475	59	000
Max.		9020	185	6560	738	000	Max.		9240	140	6716	1827	70
Mean		5098	81	4129	218	000	Mean		7086	44	4692	461	15
S.D.		±1149	±71	±1117	±283	±000	S.D.		±1762	±49	±1340	±462	±23
C. of V.		23	88	27	130	000	C. of V.		25	111	29	100	153

As both the male and female groups increased in age, the average numbers of red blood corpuscles per cu. mm. of blood increased. The greatest differences occurred in the adult groups where the average number for the males was 11,185,000 with limits of 9,648,000 to 13,264,000; the average number for the females was 11,134,000 with limits of 9,552,000 to 13,592,000.

TABLE III

NUMBERS OF THE BLOOD ELEMENTS IN RACCOON OVER THREE AND UNDER SEVEN MONTHS OF AGE

Males				Females			
No.	R.b.cs.	Hb. gm.	Ind.	No.	R.b.cs.	Hb. gm.	Ind.
1	—	7.4	—	1	9.128	8.5	0.31
2	8.240	9.0	0.37	2	8.152	8.7	0.36
3	7.320	7.7	0.36	3	8.776	7.9	0.29
4	9.344	8.7	0.32	4	—	8.5	—
5	10.360	8.1	0.27	5	7.624	9.1	0.38
6	10.872	9.5	0.29	6	7.568	9.2	0.38
7	10.408	9.9	0.33	7	11.504	8.7	0.24
8	13.296	9.5	0.24	8	9.344	7.9	0.29
9	10.400	9.4	0.31	9	11.752	8.6	0.24
10	9.192	6.3	0.23	10	11.704	8.4	0.23
11	9.464	7.7	0.28	11	10.080	7.7	0.25
12	10.400	9.3	0.31	—	—	—	—
13	9.856	7.9	0.26	—	—	—	—
Min.	7.320	6.3	0.23	Min.	7.568	7.7	0.23
Max.	13.296	9.9	0.37	Max.	11.752	9.2	0.38
Mean	*9.929*	*8.4*	*0.29*	Mean	9.563	*8.5*	*0.29*
S.D.	±1.410	±1.0	±0.04	S.D.	±1.549	±0.4	±0.18
C. of V.	*14*	*12*	*14*	C. of V.	*16*	*5*	*62*

Grams of Haemoglobin per 100 cc. of Blood

The average number of grams of haemoglobin per 100 cc. of blood for all the groups examined was, males 9.1; females 8.7 with a maximum of 12.0 for males, which occurred in an adult and in a young male between seven and twelve months of age. The maximum amount for females was 11.5 gm. which was found in an adult. The minimum for males was 5.1 gm. and for females 4.9 gm. occurring in raccoon under three months of age. The haemoglobin content followed the trend of the red blood cells, considering the groups as a whole. The amounts of haemoglobin increased with age in the male groups. In the females the group between seven and twelve months of age showed a slightly higher haemoglobin content than did the adult group. The greatest differences in haemoglobin content occurred in the groups between three and seven months of age where the average for males was 8.4 with limits of 6.3 to 9.9 and the average for females was 8.5 with limits of 7.7 to 9.2.

Color Index

The average color index for all groups examined was .30 for both males

and females. The extreme limits for males were .20 to .40; for females .20 to .39. The color index increased with age in the male groups. In the females the group between seven and twelve months of age showed a higher

TABLE IV

NUMBERS OF THE BLOOD ELEMENTS OF RACCOON OVER THREE AND UNDER SEVEN MONTHS OF AGE

Males							Females						
No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
1	17200	63.0	0.5	35.5	1.0	0.0	1	17200	77.5	0.5	20.0	1.5	0.5
		10836	86	6106	172	000			13330	86	3440	258	86
2	13000	86.5	0.0	13.0	0.5	0.0	2	10800	75.0	1.0	24.0	0.0	0.0
		11245	000	1690	65	000			8100	108	2592	000	000
3	14000	76.0	0.0	22.5	1.0	0.5	3	21400	74.0	0.0	22.0	4.0	0.0
		10640	000	3150	140	70			15836	000	4708	856	000
4	19400	69.5	0.0	28.5	2.0	0.0	4	13400	64.5	1.0	33.0	1.5	0.0.
		13483	000	5529	388	000			8643	134	4422	201	000
5	22000	50.0	1.0	46.5	2.5	0.0	5	14000	63.0	0.5	34.0	2.5	0.0
		11000	220	10230	550	000			8820	70	4760	350	000
6	14800	48.0	0.5	48.5	3.0	0.0	6	16000	85.5	0.0	11.5	3.0	0.0
		7104	74	7178	444	000			13680	000	1840	480	000
7	19400	84.0	0.0	15.0	1.0	0.0	7	29400	63.0	0.5	32.5	3.0	1.0
		16296	000	2910	194	000			18522	147	9555	882	294
8	28600	66.5	0.0	31.0	1.5	1.0	8	13200	63.5	0.0	36.5	0.0	0.0
		19019	000	8866	429	286			8382	000	4818	000	000
9	27800	57.0	0.5	42.5	0.0	0.0	9	8000	63.0	2.0	32.5	2.5	0.0
		15846	139	11815	000	000			5040	160	2600	200	000
10	14200	51.0	0.5	48.0	0.5	0.0	10	17400	76.0	1.0	19.5	3.5	0.0
		7242	71	6816	71	000			13224	174	3393	609	000
11	15800	69.0	0.0	30.0	1.0	0.0	11	28000	32.0	1.5	62.0	4.5	0.0
		10902	000	4740	158	000			8960	420	17360	1260	000
12	32000	60.0	0.5	35.0	4.0	0.5	—	—	—	—	—	—	—
		19200	160	11300	1280	160			—	—	—	—	—
13	16800	45.0	0.5	54.0	0.5	0.0	—	—	—	—	—	—	—
		7560	84	9072	84	000			—	—	—	—	—
Min.	13000	45.0	0.0	13.0	0.0	0.0	Min.	8000	32.0	0.0	11.5	0.0	0.0
Max.	32000	86.5	1.0	54.0	4.0	1.0	Max.	29400	85.5	2.0	62.0	4.5	1.0
Mean	19600	63.5	0.3	34.6	1.4	0.15	Mean	17164	67.0	0.73	29.8	2.4	0.14
S.D.	±5948	±12.8	±0.1	±12.4	±1.1	±0.9	S.D.	±6658	±11.8	±0.49	±12.8	±1.4	±0.33
C. of V.	30	20	33	36	79	600	C. of V.	39	18	67	43	58	236
Min.		7104	000	1690	000	000	Min.		5040	000	1840	000	000
Max.		19200	220	11815	1280	286	Max.		18522	420	17360	1260	294
Mean		12336	64	6877	306	40	Mean		11139	118	5408	463	35
S.D.		±3966	±70	±3132	±326	±84	S.D.		±3844	±113	±4218	±382	±86
C. of V.		32	109	46	106	210	C. of V.		34	96	71	82	246

TABLE V

NUMBERS OF THE BLOOD ELEMENTS IN RACCOON OVER SEVEN AND UNDER TWELVE MONTHS OF AGE

Males				Females			
No.	R.b.cs.	Hb. gm.	Ind.	No.	R.b.cs.	Hb. gm.	Ind.
1	13.368	9.7	0.25	1	9.120	9.7	0.36
2	11.368	12.0	0.36	2	10.352	9.5	0.32
3	11.816	9.5	0.29	3	10.424	11.2	0.37
4	12.344	11.9	0.33	4	9.648	10.5	0.39
5	10.400	9.4	0.31	5	10.928	11.0	0.37
—	—	—	—	6	11.344	10.8	0.33
Min.	10.400	9.4	0.25	Min.	9.120	9.5	0.32
Max.	13.368	12.0	0.36	Max.	11.344	11.2	0.39
Mean	11.799	10.5	0.31	Mean	10.303	10.5	0.36
S.D.	±0.990	±1.2	±0.037	S.D.	±0.743	±0.6	±0.06
C. of V.	8	11	12	C. of V.	7	6	17

TABLE VI

NUMBERS OF THE BLOOD ELEMENTS IN RACCOON OVER SEVEN AND UNDER TWELVE MONTHS OF AGE

Males							Females						
No.	*W.b.cs.*	*Ly.*	*Mo.*	*Ne.*	*Eo.*	*Ba.*	*No.*	*W.b.cs.*	*Ly.*	*Mo.*	*Ne.*	*Eo.*	*Ba.*
1	35000	45.5	3.0	45.5	5.0	1.0	1	36200	48.0	4.0	42.0	5.0	1.0
		15925	1050	15925	1750	350			17376	1448	15204	1810	362
2	24200	56.5	4.0	38.5	1.0	0.0	2	28000	57.0	2.0	38.5	2.5	0.0
		13673	968	9317	242	000			15960	560	10780	700	000
3	31000	37.0	1.0	56.5	5.5	0.0	3	28600	53.5	1.5	42.0	3.0	0.0
		11470	310	17515	1705	000			15301	429	12012	858	000
4	27800	54.0	3.0	39.5	3.5	0.0	4	19800	54.5	1.0	43.5	1.0	0.0
		15012	834	10981	973	000			10791	198	8613	198	000
5	22600	60.0	1.0	36.5	2.5	0.0	5	22400	42.5	1.0	52.0	4.0	0.5
		13560	226	8249	565	000			9520	224	11648	896	112
—	—	—	—	—	—	—	6	24600	46.0	3.5	48.5	2.0	0.0
		—	—	—	—	—			11316	861	11931	492	000
Min.	22600	37.0	1.0	36.5	1.0	0.0	Min.	19800	42.5	1.0	38.5	1.0	0.0
Max.	35000	60.0	4.0	56.5	5.5	1.0	Max.	36200	57.0	4.0	52.0	4.0	1.0
Mean	28120	50.6	2.4	44.1	3.5	0.2	Mean	26600	50.3	2.2	44.4	2.9	0.3
S.D.	±4507	±8.3	±1.2	±7.2	±1.6	±0.4	S.D.	±5260	±5.1	±1.2	±4.5	±1.3	±0.9
C. of V.	16	16	50	16	46	200	C. of V.	20	10	5	10	45	300
Min.		11470	226	8249	242	000	Min.		9520	198	8613	198	000
Max.		15925	1050	15925	1750	350	Max.		17376	1448	15204	1810	362
Mean		13928	677	12397	1047	70	Mean		13377	620	11698	824	79
S.D.		±1510	±342	±3670	±602	±140	S.D.		±2829	±432	±1949	±499	±133
C. of V.		11	51	30	57	200	C. of V.		21	70	17	61	168

color index than did the adults. An index of .35 was shown for the group between seven and twelve months and of .31 for the adult group. The greatest differences occurred in the group between three and seven months of age where the average for the males was .29 with limits of .23 to .37; the average for the females was .29 with limits of .24 to .38.

White Blood Corpuscles

The average number of white blood corpuscles for all groups examined was 17,880 for males, with a maximum number of 35,000 occurring in a raccoon between seven and twelve months of age and a minimum of 6,800 in a raccoon under three months of age. The average number of white blood

TABLE VII

NUMBERS OF THE BLOOD ELEMENTS IN ADULT RACCOON

Males				Females			
No.	R.b.cs.	Hb. gm.	Ind.	No.	R.b.cs.	Hb. gm.	Ind.
1	13.264	11.2	0.29	1	13.496	11.5	0.29
2	12.424	12.0	0.33	2	10.344	10.5	0.35
3	11.160	11.0	0.33	3	11.896	10.8	0.30
4	10.000	11.9	0.40	4	11.552	11.0	0.31
5	9.648	11.2	0.37	5	9.552	9.7	0.36
6	10.616	11.9	0.36	6	9.928	11.0	0.37
—	—	—	—	7	10.592	8.2	0.25
—	—	—	—	8	10.384	10.2	0.34
—	—	—	—	9	10.008	10.0	0.33
—	—	—	—	10	13.592	11.0	0.26
Min.	9.648	11.0	0.29	Min.	9.552	8.2	0.25
Max.	13.264	12.0	0.40	Max.	13.592	11.5	0.37
Mean	11.185	11.5	0.35	Mean	11.134	10.4	0.31
S.D.	±1.281	±0.4	±0.03	S.D.	±1.381	±0.89	±0.13
C. of V.	11	3	8.6	C. of V.	12	9	42

cells found in female raccoon was 18,166, with a maximum number of 36,200 occurring in a raccoon between seven and twelve months of age. The minimum number of 6,600 occurred in a raccoon under three months of age. The greatest differences occurred in the groups between seven and twelve months of age. The highest numbers of white blood cells per cu.mm. of blood occurred in the groups between seven and twelve months of age. In this group the average number for the males was 28,120 with limits of 22,600 to 35,000; the average number for the females was 26,600 with limits of 19,800 to 36,000. The lowest number of white blood cells per cu. mm. of blood was found in the groups under three months of age. In this group the average number for the males was 9,525 with limits of 6,800 to 16,400, and the average number for the females was 12,850 with limits of 6,600 to 19,000.

Lymphocytes

The average number of lymphocytes for the series of male groups was 7,715 (58.6%) cells per cu. mm. of blood and for the female groups 9,419

(56.1%). The maximum number of lymphocytes found in the male groups was 19,200 (60.0%) in a raccoon between three and seven months of age, and the minimum was 3,640 (53.0%) found in a raccoon under three months of age. The maximum number for the female groups was 18,522 (63.0%) found in a raccoon between three and seven months of age, and the minimum number was 3,960 (60.0%) found in a raccoon under three months of age. The highest average number of lymphocytes per cu. mm. of blood for the groups was, males 13,928 (50.6%) found in the group between seven and twelve months of age, with limits of 11,470 (37.0%) to 15,925 (60.0%); females 11,377 (50.3%) which was also found in the group between seven

TABLE VIII

NUMBERS OF THE BLOOD ELEMENTS IN ADULT RACCOON

Males							Females						
No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	No.	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
1	12200	78.5	0.5	19.5	1.5	0.0	1	26800	46.0	3.5	46.5	4.0	0.0
		9577	61	2379	183	000			12328	938	12462	1072	000
2	14800	59.0	2.0	37.5	1.5	0.0	2	16200	54.5	1.0	43.0	1.5	0.0
		8732	296	5550	222	000			8829	162	6966	243	000
3	15600	68.5	1.0	23.5	7.0	0.0	3	15000	40.5	0.5	57.5	1.5	0.0
		10686	156	3666	1092	000			6075	75	8625	225	000
4	13000	63.0	2.0	30.0	5.0	0.0	4	18600	65.5	0.0	30.5	4.0	0.0
		8190	260	3900	650	000			12183	000	5673	744	000
5	14000	72.0	2.0	23.0	3.0	0.0	5	12400	35.0	0.0	60.5	4.5	0.0
		10080	280	3220	420	000			4340	000	7502	558	000
6	16200	60.0	0.0	33.5	6.5	0.0	6	13400	52.0	0.0	43.0	5.0	0.0
		9720	000	5427	1053	000			6968	000	5762	670	000
—	—	—	—	—	—	—	7	13600	—	—	—	—	—
		—	—	—	—	—			—	—	—	—	—
—	—	—	—	—	—	—	8	14400	53.0	0.0	39.0	8.0	0.0
		—	—	—	—	—			7632	000	5616	1152	000
—	—	—	—	—	—	—	9	20000	43.0	1.0	50.0	6.0	0.0
		—	—	—	—	—			8600	200	10000	1200	000
—	—	—	—	—	—	—	10	10600	54.0	1.0	41.0	4.0	0.0
		—	—	—	—	—			5724	106	4346	424	000
Min.	12200	59.0	0.0	19.5	1.5	0.0	Min.	10600	35.0	0.0	30.5	1.5	0.0
Max.	16200	78.5	2.0	37.5	7.0	0.0	Max.	26800	65.5	3.5	60.5	8.0	0.0
Mean	14300	66.8	1.25	27.8	4.1	0.0	Mean	16100	49.3	0.8	45.7	4.3	0.0
S.D.	±1398	±6.9	±0.8	±6.3	±2.2	±0.0	S.D.	±4416	±8.1	±1.0	±8.2	±1.9	±0.0
C. of V.	10	10	64	23	54	000	C. of V.	27	16	125	18	44	000
Min.		8190	000	2379	183	000	Min.		4340	000	4346	225	000
Max.		10686	296	5550	1092	000	Max.		12328	938	12462	1200	000
Mean		9498	176	4024	603	000	Mean		8075	165	7439	699	000
S.D.		±1816	±113	±1135	±365	±000	S.D.		±2597	±282	±2409	±354	±000
C. of V.		19	64	28	61	000	C. of V.		32	171	32	51	000

and twelve months of age, with limits of 9,520 (42.5%) to 17,376 (57.0%). In the raccoon groups under one year of age the total number of lymphocytes per cu. mm. of blood tends to increase with the age of the animal, and decreases after the adult age is reached.

Monocytes

The average number of monocytes occurring in the blood of male raccoon of all the groups examined was 249 (1.2%) with extreme limits of 0 to 1,050 (3.0%). The average for females was 237 (1.3%) with limits of 0 to 1,448 (4.0%). The highest average number of monocytes for a group was found in the groups between seven and twelve months of age where the average for the males was 677 (2.4%) with limits of 226 (1.0%) to 1,050 (4.0%); the average for the female group was 620 (2.2%) with limits of 198 (1.0%) to 1,448 (4.0%).

Neutrophiles

The average number of neutrophiles for male raccoon groups examined was 12,397 (44.1%); and for the females 11,698 (44.4%). The extreme limits for the males were 1,690 (13.0%) to 15,925 (45.5%). Limits for females were 1,840 (11.5%) to 15,204 (42.0%). The highest average number found in a group was in the raccoon between seven and twelve months of age, where the average number for the male group was 12,397 (44.1%) with limits of 8,249 (36.5%) to 15,925 (56.5%); the average for the female group was 11,698 (44.4%) with limits of 8,613 (38.5%) to 15,204 (52.0%). The lowest average number of neutrophiles occurring in the male groups was in the adults, where 4,024 (27.8%) with limits of 183 (19.5%) to 1,092 (37.5%) were found. In the female groups, the lowest number was found in the group under three months of age where the average was 7,309 (39.8%) with limits of 4,692 (29.8%) to 11,698 (45.7%).

TABLE IX

FINAL MEANS FOR THE BLOOD ELEMENTS IN ALL GROUPS OF RACCOON

Males				Females			
Age, months	R.b.cs.	Hb. gm.	Ind.	Age, months	R.b.cs.	Hb. gm.	Ind.
0–3	8.384	6.0	0.23	0–3	7.995	5.5	0.23
3–7	9.929	8.4	0.29	3–7	9.563	8.5	0.29
7–12	11.799	10.5	0.32	7–12	10.303	10.5	0.35
Adults	11.185	11.5	0.35	Adults	11.134	10.4	0.31
Min.	8.384	6.0	0.23	Min.	7.995	5.5	0.23
Max.	11.799	11.5	0.35	Max.	11.134	10.5	0.35
Mean	10.324	9.1	0.30	Mean	9.749	8.7	0.30
S.D.	±1.307	±2.1	±0.08	S.D.	±1.155	±2.0	±0.04
C. of V.	13	23	27	C. of V.	12	23	13

Eosinophiles

The average number of eosinophiles occurring in the blood of male raccoon of all the groups examined was 544 (2.7%) with limits of 218 (1.4%) to 1,047 (4.1%). The average for females was 611 (3.3%) with limits of 458 (2.4%) to 824 (4.3%). The highest average number for a group was found in the group between seven and twelve months of age where the average for the male group was 1,047 (3.5%) with limits of 242 (1.0%) to 1,750 (5.5%). The average number for the female group was 824 (2.9%) with limits of 198 (1.0%) to 1,810 (4.0%). The lowest average number for a group was found in the raccoon under three months of age, where the average for the male group was 218 (1.9%) with limits of 68 (1.0%) to 738 (4.5%); the average number for the female group was 461 (3.4%) with limits of 59 (0.5%) to 1,827 (10.5%).

Basophiles

The average number of basophiles per cu. mm. of blood for all groups examined was, males 28 (0.1%) with limits of 0 to 70 (0.2%); females 32 (0.1%) with limits of 0 to 79 (0.3%). The highest average number of basophiles for a group was found in raccoon between seven and twelve months of age. In this group the average number for the males was 70 (0.2%) with limits of 0 to 350 (1.0%); the highest average number for the females was

TABLE X

FINAL MEANS FOR THE BLOOD ELEMENTS IN ALL GROUPS OF RACCOON

Males							Females						
Age, months	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.	Age, months	W.b.cs.	Ly.	Mo.	Ne.	Eo.	Ba.
0–3	9500	53.5	0.9	44.0	1.0	0.0	0–3	12900	58.0	0.4	38.2	3.4	0.13
		5098	81	4129	218	000			7086	44	4692	458	15
3–7	19600	63.5	0.3	34.6	1.4	0.2	3–7	17164	67.0	0.7	29.8	2.4	0.14
		12336	64	6877	306	40			11139	118	5408	463	35
7–12	28120	50.6	2.4	44.1	3.5	0.2	7–12	26600	50.3	2.2	44.4	2.9	0.3
		13928	677	12397	1047	70			11377	620	11698	824	79
Adults	14300	66.8	1.3	27.8	4.1	0.0	Adults	16000	49.3	0.8	45.7	4.3	0.0
		9498	*176*	*4024*	*603*	*000*			*8075*	*165*	*7439*	*699*	*000*
Min.	9500	50.6	0.3	27.8	1.4	0.0	Min.	12900	49.3	0.4	29.8	2.4	0.0
Max.	28120	66.8	2.4	44.1	4.1	0.2	Max.	26600	67.0	2.2	45.7	4.3	0.3
Mean	17880	58.6	1.2	37.6	2.7	0.1	Mean	18166	56.1	1.3	39.8	3.3	0.14
S.D.	±6908	±7.1	±1.5	±6.9	±1.1	±0.1	S.D.	±5112	±14.2	±0.7	±6.3	±0.7	±0.1
C. of V.	39	12	125	18	41	100	C. of V.	28	25	54	16	21	71
Min.		5098	64	4024	218	000	Min.		7086	44	4692	458	000
Max.		13928	677	12397	1047	70	Max.		11377	620	11698	824	79
Mean		7715	249	6857	544	28	Mean		9419	237	7309	611	32
S.D.		±4183	±250	±3360	±324	± 30	S.D.		±1874	±225	±2727	±157	±30
C. of V.		54	100	48	60	107	C. of V.		20	95	37	26	94

79 (0.3%) with limits of 0 to 362 (1.0%). Basophiles were not observed in the blood of any of the raccoon in either the male or female adult groups, or in the male group under three months of age. In all the groups examined there were a number of raccoon in whose blood basophiles were not observed.

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OSCILLATIONS IN THE MAGNETO-IGNITION SYSTEM AND THEIR ELIMINATION[1]

By Wilbert B. Smith[2]

Abstract

A theoretical analysis of the magneto-ignition system operated in air shows that there are 10 possible oscillations, but an oscillographic analysis shows that in the range tested there are only two actually present, one of frequency about 3500 cycles per second and the other about 180×10^6 cycles per second; the latter is responsible for most of the radio interference. Series resistances, "suppressors", were found to reduce the interference and, when combined with good shielding, very nearly eliminated it, but when large values of series resistance were used the brilliancy of the spark was greatly reduced. This was overcome by increasing the electrostatic capacity of the spark plug by a special design, so that, when the plug was used with a 50,000 ohm suppressor and adequate shielding, there was negligible interference with only a slight reduction in the brilliancy of the spark in air.

Introduction

The high-tension magneto-ignition system is essentially a device for changing mechanical energy into heat energy and releasing it at a certain predetermined time. Although the principle of operation of the system is relatively simple, a complete analysis is extremely complicated owing to the many uncertain factors which enter into its performance.

The successful operation of any ignition system involves sudden changes of currents and voltages, hence a great variety of transient oscillations is possible. It is the purpose of this paper to give special attention to these oscillations and in particular to those responsible for radio interference.

General Description

The constants of the magneto used in these investigations are given in Table I. It was driven normally at 900 r.p.m. through a gear train from the synchronous motor which operated the timing contacts for a General

TABLE I

Circuit constants

Quantity	Value	Unit	Remarks	Quantity	Value	Unit	Remarks
N_1	273	Turn	—	C_2	100	μμf.	Value at 3500 cycles per sec.
L_1	0.02	Henry	Practically constant				
R_1	0.11	Ohm	D-c. resistance		65	μμf.	Value at 25×10^4 cycles per sec.
C_1	0.041	μf.	Mica condenser				
K	0.97		Computed from resonance	C	5	μμf.	AC spark plug
M	0.75	Henry	Computed from resonance		10	μμf.	Oleo spark plug
N_2	13700	Turn	—	L	10^{-6}	Henry	Computed approximately
L_2	30	Henry	Practically constant	a	3600	Volt	AC spark plug
R_2	3000	Ohm	D-c. resistance	b	5×10^3	Ohm	Estimated approximately with oscillograph
	7×10^4	Ohm	Effective resistance at 3500 cycles per sec.				

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Electric cathode-ray oscillograph. Leads from the magneto to the spark plug were of ordinary ignition cable except where otherwise noted. The spark plugs used were: an AC automobile plug, an Oleo aviation plug, and a special plug to be described later.

Low-frequency oscillograms were taken on a Westinghouse six element oscillograph, and high-frequency traces were taken on a G.E. cathode-ray oscillograph—directly when possible or by the judicious use of vacuum tube amplifiers when either the voltages or currents were beyond the range of the instruments.

Other apparatus included a Burt cathode-ray oscilloscope with which a visual study of the wave forms was made, a three tube radio set used for monitoring the interference, and miscellaneous apparatus used in determining the magneto constants.

The electrical circuit is shown diagrammatically in Fig. 1. This circuit represents conditions prevailing to a fair degree of accuracy providing that due allowance be made for the variation of circuit constants with current or frequency. Most of these quantities remained practically constant over the range of operation, with the exception of the distributed capacity C_2, the secondary effective resistance R_2, and the spark resistance r. The distributed capacity C_2 was measured by a substitution method and was found to decrease slightly at higher frequencies, having the value 100 $\mu\mu$f. at 3500 cycles per second and 65 $\mu\mu$f. at 25×10^5 cycles per second. The secondary effective resistance was also measured by a substitution method and was found to increase enormously with frequency. These results are plotted in Fig. 2.

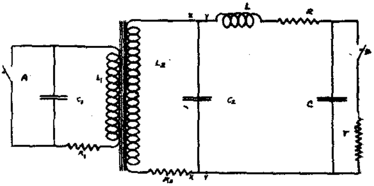

FIG. 1. *Equivalent electrical circuit of the high-tension magneto-ignition system.*

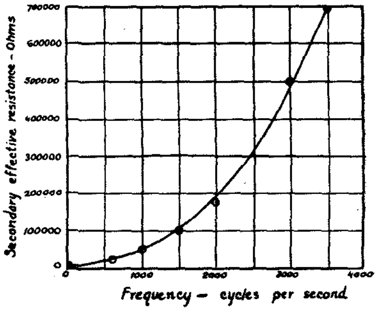

FIG. 2. *Curve showing the variation of secondary effective resistance, R_2, with frequency.*

It was practically impossible to measure the effective spark resistance r owing to its erratic behavior. Therefore, the semi-

empirical formula for the voltage drop across a spark discharge (3),

$$e = a + bi$$

was used, where e is the voltage drop, i the current, and a and b are constants, not necessarily the same values throughout the discharge. Numerical values were used which seemed to be in agreement with experiment.

Table I gives a list of the circuit constants determined for the armature position immediately following the opening of the timing contacts.

Operation

The production of each spark involves a cycle of operations, which for the convenience of analysis and reference may be divided into six rather distinct periods (2), as follows:

1. During the first period the low-voltage winding or primary is short-circuited by the timing contacts, and as the armature rotates, a certain current, I, is generated in the coil. This current stores in the magnetic circuit $\frac{1}{2} LI^2$ joules of energy which later is used to supply current to the spark plug, together with the incidental losses.

2. A few microseconds before a spark is desired at the plug, a cam opens the timing contacts, thus breaking the primary circuit. The primary current decreases rapidly, inducing high voltages in both primary and secondary windings. The high secondary voltage charges the stray secondary capacities to a sufficiently high potential to break down the resistance of the gas between the spark plug electrodes.

3. This period consists of the discharge of the stray secondary capacities through the leads, which are inductive, and the gap. Since the values of inductance and capacity are usually small, and unless suppressors are used, the resistance is also small, oscillations of a very high frequency may take place.

4. Following the discharge of Period 3, the remaining energy in the magnetic and electric circuits is dissipated in the spark between the electrodes of the spark plug.

5. Owing to high speeds or improper adjustments of the timing contacts, it is possible that the latter may close before all the energy has been dissipated in the spark, in which case the energy may divide between the spark and the primary circuit.

6. The circuit remains substantially dead for a considerable time, until the armature has rotated sufficiently for the cycle to begin over again for the production of the next spark.

Period 1

As the magneto armature rotates, the magnetic flux linking the windings changes and induces rotational e.m.f.'s in them. Since the purpose of these e.m.f.'s is to establish a current I in the short-circuited primary prior to the opening of the timing contacts, it follows that, in a well designed magneto, there is practically no rotational voltage for the greater part of the cycle, but just prior to the opening of the timing contacts, the rotational e.m.f.

rises rather suddenly to quite a large value. The oscillogram in Fig. 4 shows the rotational voltage for the magneto used in these tests. The peak value on this wave corresponds to a primary voltage of about 13.5 volts. The lower trace is that of a 60-cycle timing wave.

Period 2

When a spark is desired at the plug, the timing contacts are separated by a cam, thus breaking the primary circuit. Referring to Fig. 1 (omitting all to the right of YY), it is seen immediately that the circuit becomes that of the simple induction coil. From the theory of the induction coil, it follows that there may be two possible frequencies of oscillation if the decrement of the circuit is low, or only one oscillation and one hyperbolic if the decrement is higher, or only hyperbolics if the decrement is very high. Taking the most favorable case, that of low decrement, there will be two frequencies of oscillation which we may call f_1 and f_2, whose numerical values will depend on the circuit constants, chiefly L_1, C_1, L_2, and C_2. From an inspection of the numerical values of these quantities, it is evident that f_1 and f_2 will be of audio frequency, that is, less than 16×10^3 cycles per second.

In the magneto under test, it was found that only one oscillation and one hyperbolic, both with high decrement, occurred. The open-circuit secondary voltage wave is shown in Fig. 9, as taken with the vibrator oscillograph, and in Fig. 8 as taken with the cathode-ray oscillograph. The initial peak value of these waves corresponds to about 9,000 volts. As may be seen (Fig. 8), there is initially an oscillation of large amplitude, frequency 3500 cycles per second and decrement 0.83×10^4, which is followed by a unidirectional component which decreases much more slowly. There were no radio-frequency oscillations of observable magnitude on open circuit.

Period 3

When the voltage across the sparking tips has built up to a sufficient value, the gap breaks down and the stray secondary capacities discharge through the gap. Referring to Fig. 1, breakdown of the gap is the same as closing the switch B. Consider first the circuit consisting of r, C, R, and L (assuming that C_2 acts as a virtual short circuit for high-frequency oscillations). By equating voltages around the circuit, the operational solution of this circuit becomes (1)

$$i = \frac{(E-a)(1+CRP+LCP^2)}{(R+b)+(bRC+L)P+bLCP^2}\,\mathbf{1}\,.$$

A possible general solution of this equation is of the form

$$i = Ae^{-\alpha t}\sin(\beta t-\phi),$$

where β has the value given by

$$\beta = \sqrt{\frac{(bRC-L)^2}{(2bLC)^2} - \frac{(R+b)}{bLC}}\,.$$

This is a damped oscillation of frequency $f_3 = \beta/2\pi$. If we substitute typical numerical values in the above equation we find that f_3 is of the order of 7×10^7 cycles per second, which corresponds to a wave-length of about four metres.

Consider next the circuit consisting of r, R, L, and C_2 (assuming that C is negligibly small). The operational solution in this case is,—

$$i = \frac{(E-a)C_2P}{1 + (bC_2 + RC_2)P + LC_2P^2} \mathbf{1},$$

and a general solution is,

$$i = Ae^{-\alpha t} \sin (\beta t - \phi),$$

where β has the value given by

$$\beta = \sqrt{\frac{(b+R)^2}{(2L)^2} - \frac{1}{LC_2}}.$$

This also is a damped oscillation of frequency $f_4 = \beta/2\pi$, which, when suitable numerical values are substituted, is of the order of 2×10^7 cycles per second.

Because of the ultra high frequency of f_3 and f_4, it was impossible to obtain oscillograms of them. However, by means of the radio set, it was possible to confirm the presence of one or both, but it was not possible to determine their exact frequency or magnitude, as both of these characteristics varied considerably. Suffice it to say that the frequencies were of the order indicated above, and that the magnitudes were small compared with that of the f_{10} oscillation of the spark.

When the gap breaks down, there are in addition to the above, transients due to a voltage E applied at the switch B to the circuit consisting of r in series with C_2 and Z in parallel, where Z is the impedance operator for all to the left of XX, Fig. 1. The operational solution in this case works out to be,

$$i = \frac{(C_2PZ + 1)E}{C_2bZP + b + Z} \mathbf{1}.$$

A general solution of the above equation may contain two damped oscillations of frequencies f_5 and f_6. From an inspection of the circuit constants involved, it follows that these two frequencies, when present, will be of the same order of magnitude as f_1 and f_2, namely, audio frequency. In the actual ignition system used in these tests, it was found that no oscillations of this nature took place, those transients which were present being entirely hyperbolic.

Period 4

After the breakdown of the spark gap, with its associated impulse transients, there follows a "steady state", which persists until the energy available is dissipated in the gap. This steady state is characterized by either a steady current flow or a continuous oscillation which is caused by the inherent instability of a spark discharge. The latter oscillation may be called f_{10},

and is shown in Fig. 5, which is a cathode-ray oscillogram of the current flowing through the spark plug, taken by amplifying the voltage drop across a 60 ohm series resistance.

The general shape of the current wave is shown in Fig. 7, which is a low-frequency trace of the current flowing through the AC plug. An extensive visual study of this wave was made with the cathode-ray oscilloscope, and the following points were noted: The current rose suddenly to about 50 ma. when an oscillation of frequency greater than 50 kc. took place (the resolving power of the instrument was about 50 kc.). Following this oscillation the current slowly increased to about 65 ma. maximum, after which it decreased rather rapidly to zero. There was no observable discontinuity or oscillation when the current ceased.

During this period the voltage drop across the spark remained substantially constant. Fig. 10 is a low-frequency trace of the voltage across the plug during sparking. This trace was obtained by operating a UX 245 vacuum tube from a voltage divider across the plug and sending the plate current of the tube directly through the oscillograph element. The excitation of the tube was sufficiently low for its operation to be linear over the operating range. The cathode-ray oscillograph, however, showed this voltage wave to carry a "modulation" corresponding to f_{10}, which caused a variation of 100% in its magnitude.

Referring again to Fig. 5, the oscillation f_{10} appears to be without decrement and is certainly far from sinusoidal. The current appears to jump suddenly to a maximum value and then die out exponentially, the whole cycle being repeated about 180,000 times per second. Altering the external circuit constants seemed to have little effect on either the frequency or amplitude of this oscillation. The only quantities which did seem to influence it were the current flowing and the dimensions of the gap. A reduction of current produced a reduction of oscillation and a decrease of frequency, while a change in gap length produced only a change in frequency. Sphere-gap electrodes seldom gave oscillations of this nature unless the current was abnormally high. Fig. 11 is a cathode-ray oscillogram of the currents flowing in the Oleo plug and in the AC plug. Both of these traces are to the same scale and with the plugs working under identical conditions. The top trace, which is of the Oleo plug, does not show any oscillation of the nature of f_{10}, although such oscillations were occasionally observed visually.

Period 5

It is possible that the timing contacts may close while there is still an appreciable voltage across them, in which case this sudden change of circuit constants may result in further transients. If the total voltage across the timing contacts at the instant of closing be E, the effect is the same as applying $-E$ to the switch A and omitting the condenser C_1 from the circuit in Fig. 1. Considering first the case where there is no spark in progress at the instant

of closing,—that is, all to the right of YY is omitted—the operational solution for e_s, the secondary voltage, may be shown to be

$$e_s = \frac{(R_2 + L_2P)(MC_2P^2)E}{(R_1 + L_1P)(1 + R_2C_2P + L_2C_2P^2) - M^2C_2P^3} 1 .$$

One form of the general solution for this operational solution will be,

$$e_s = Ae^{-\alpha t} + A_1e^{-\alpha_1 t} \sin (\beta_1 t - \phi),$$

which indicates a single possible oscillation of frequency $f_9 = \beta_1/2\pi$. An inspection of the circuit constants shows that, should this oscillation occur, it will most probably be of audio frequency. Actually, in the magneto under test, it was not possible by any normal adjustment or speed variation to cause the contacts to close while there was sufficient voltage across them to produce an observable transient.

If there is a spark in progress when the timing contacts close, then we have the quantity $a+bi$ across the secondary of the magneto. Now, since $a+bi$ is found to be a constant for low frequencies, b must be zero (practically) and hence, if any oscillations were to take place, they would immediately be damped out.

Period 6

If there is a sudden change of voltage or current at the extinction of the spark, a further set of transients may be set up. If the current flowing at the extinction of the spark be I, the mathematical analysis becomes identical with that of the simple induction coil with, of course, the interchange of primary and secondary constants. A general solution of the resulting differential equation may contain two oscillations, which we may call f_7 and f_8, or corresponding hyperbolic terms. These frequencies will of necessity be of the same order of magnitude as f_1 and f_2.

It was found experimentally that, at the extinction of the spark, the current more often died away gradually rather than suddenly as was assumed above. In this case, since the current at extinction is zero, it follows at once that the oscillations f_7 and f_8 will be entirely absent. Referring to Fig. 10, the voltage at the extinction of the spark is seen to increase slightly as the current drops to very low values, after which it follows closely the corresponding portion of the open-circuit curve.

The oscillations appearing in Figs. 9 and 10 are due more to the under-damping of the oscillograph vibrator than to oscillations in the electrical circuit, for no oscillations approaching these amplitudes were observed with the cathode-ray instruments.

The remainder of Period 6 is characterized by the small rotational voltages prior to the repetition of the cycle.

Radio Interference

There are three ways in which a magneto-ignition system may cause interference. First, oscillations and wave forms of audio frequency may affect the radio set by induction or conduction. Second, oscillations of radio frequency may radiate sufficient energy to affect the radio-frequency circuits of the set. Third, extremely sharp wave forms may radiate pulses which may also affect the radio-frequency circuits. Of these, the last two are most important since the radio set is located usually so far from the ignition system that induction is negligible, and paths of conduction are usually absent.

The ideal remedy for any interference is, of course, to eliminate the source. In the case of oscillations due to stray inductances and capacities this is quite feasible, but oscillation of the spark and sharp wave forms offer considerably more difficulty in that there are no equations for these phenomena from which the limiting conditions may be computed.

In the section entitled "Period 3" were developed equations for the two possible impulse oscillations, f_3 and f_4. From an inspection of these equations it is readily seen that for oscillation to take place, the β terms must be imaginary. Hence for non-oscillation these terms must be real: from this we may obtain the limiting conditions.

For f_3 to be absent,

$$R \geqslant 2\sqrt{\frac{L}{C}} + \frac{L}{bC}$$

and for f_4 to be absent,

$$R \geqslant 2\sqrt{\frac{L}{C_2}} - b \quad .$$

The above relations justify the use of series resistances commercially known as suppressors, as a means of reducing radio interference. From the above equations, suppressor resistances need only be a few hundred ohms, but it is found in the present case that for satisfactory operation a resistance of about 50,000 ohms is required.

As regards sharp wave forms, very little can be done from a theoretical standpoint. Sharp current waves could be smoothed out by inserting inductance into the circuit, that is, by increasing L. Sharp voltage waves could best be dealt with by careful shielding of all apparatus subjected to these surges.

Since oscillation of the spark, f_{10}, is apparently due only to conditions in the spark, it is evident that we can eliminate this oscillation only by altering these conditions. Unfortunately the only conditions over which there is direct control are the current flowing and the dimensions of the gap, the others being either dependent on these or fixed by conditions of operation.

For the purpose of monitoring the radio interference, use was made of a three tube, 30 to 500 metre radio set, located approximately six feet away from the ignition system. When the ignition system was functioning normally

the interference was so bad that local broadcasting stations were completely drowned out. The intensity of the interference was almost constant over the complete range of the set, being only slightly greater on the shorter waves. There was no noticeable interference when the magneto was operated on open circuit.

A variable resistance was connected in series with the spark plug and its value varied from zero to 50,000 ohms. The interference was observed to decrease steadily as the resistance was increased until it nearly vanished at a value of 40,000 ohms. Increasing the resistance still further had no noticeable effect until a value was reached such that a spark no longer occurred. With no resistance in series the spark was brilliantly white, but as resistance was inserted it became blue and less brilliant until at 50,000 ohms it was a feeble blue line.

A study of the wave forms, made with the cathode-ray oscilloscope, led to the conclusion that the suppressor did not reduce interference by virtue of its damping action but by the great reduction in current which its use involved, the peak current being only about 6 ma. When the original current, peak 65 ma., was forced through the spark by means of higher voltages, the interference was as bad as ever.

Fig. 12 shows the action of a 50,000 ohm suppressor on the oscillations occurring with the AC spark plug. The lower trace is of the amplified voltage drop across a 60 ohm series resistance without the suppressor in the circuit, and shows an oscillation of the spark similar to that appearing in Fig. 5. The upper trace is of the same voltage drop but with a 50,000 ohm suppressor in series. As may be seen, the suppressor has eliminated the oscillation appearing on the lower trace, but there is still present a very small oscillation of frequency about 45 kc. A visual study gave precisely the same results for the Oleo plug.

A concentric shielded cable was tried as a lead from the magneto to the spark plug and this effected a considerable reduction in the interference, but, owing to the unshielded portions at the ends, some energy was still radiated. It was found that as little as an inch of unshielded cable was sufficient to cause objectionable interference. However, when this lead was used with a suppressor at the plug, the interference dropped to a negligible quantity, even though a portion of the cable remained unshielded.

Design of the Special Spark Plug

It appears from the preceding experimental investigations that the radio interference arising from the spark plug spark may be reduced to a satisfactory level, *if not eliminated completely, by the use of a 50,000 ohm suppressor* resistance in series with the plug, combined with complete shielding.

However, there is the objection to the use of a high resistance in series with the spark that the efficiency of the spark may thereby be reduced. It may be shown mathematically and confirmed experimentally, that the cross-section

area of a spark is closely proportional to the current flowing through it, hence a reduction in current causes a proportionate reduction in the size of the spark. With the suppressor in circuit the maximum current is never much greater than the mean current, hence the size of the spark is more or less determined by this mean current. Without the suppressor there is an initial rush of current, which may be much larger than the mean current, owing to the discharge of the secondary stray capacities through the gap. This large initial current will result in one or both of two things, the area of the spark will be initially large or the temperature will be high, either effect making for a greater efficiency of the spark.

If now a condenser of a value equal to the stray secondary capacities be connected across the spark plug between the suppressor and the gap, it is apparent that the initial conditions will be very nearly the same as without the suppressor. That is, the condenser will charge up through the suppressor resistance until the voltage becomes high enough to break down the gap, whereupon it will discharge through the gap with a current at least as great as that due to the discharge of the stray secondary capacities. This initial current will be followed by the usual small current which the suppressor permits to flow.

With the above considerations in mind, the spark plug shown in section in Fig. 3 was constructed. The right-hand portion is the regulation spark plug base, with the exception of the sphere-gap electrodes. The left-hand portion contains the built in condenser, which consists of a stack of alternate large and small aluminium washers separated by mica washers. The core is of mica and the entire assembly is held together by collars threaded on the central rod. It was decided to use spherical electrodes, since it was found that oscillation of the spark seldom occurred with this type of electrode when the current was of the order found in actual operation.

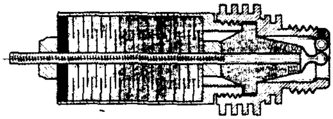

Fig. 3. *Sectional drawing of special spark plug, showing built-in condenser and sphere-gap electrodes.*

Fig. 6 is a cathode-ray oscillogram of the current flowing through this special plug. The upper trace is of the current flowing without the suppressor in the circuit, and the lower one is the same but with a 50,000 ohm suppressor in series. These traces are of the amplified voltage drop across a 60 ohm series resistance. As may be seen, there is at no time a serious oscillation of the spark; the transient which does appear on the upper trace is greatly reduced on the lower one when the suppressor is in circuit. (Compare with Figs. 5, 11, and 12.)

The spark supplied by the special plug was typical of the discharging of a condenser, being brilliantly white and accompanied by the usual snap. There was only a very slight decrease in brilliancy when the suppressor was inserted

in the circuit. The radio interference from this plug was almost as bad as that from the other plugs when no suppressor was used, but with it the interference dropped to a negligible value, and when a shielded cable was used also, vanished entirely.

Acknowledgment

In conclusion the writer wishes to express his gratitude to Dr. H. Vickers, Head of the Department of Electrical Engineering at the University of British Columbia, for his many helpful suggestions and constructive criticisms.

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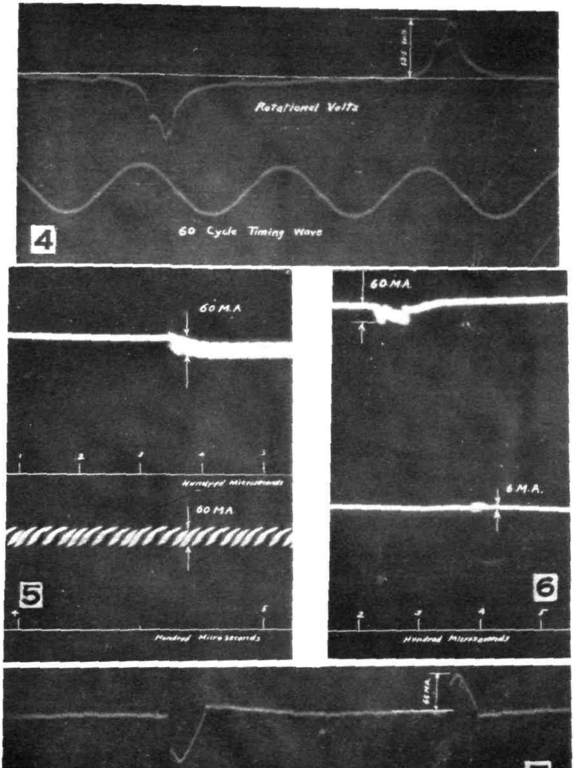
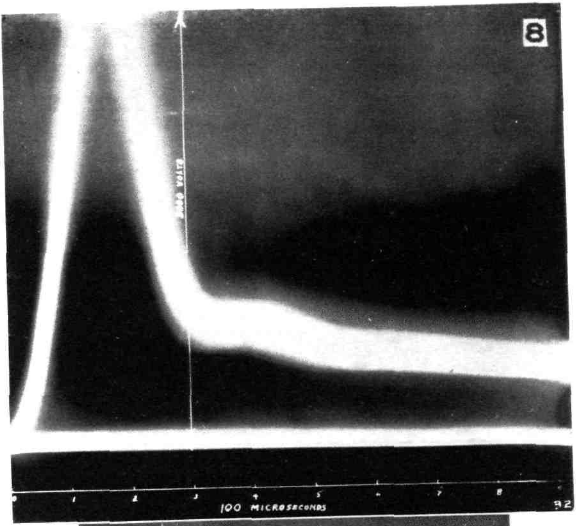


FIG. 4. *Rotational voltage of the magneto.* FIG. 5. *Cathode-ray oscillogram of current flowing through spark plug, showing oscillation of the spark f_{10}.* FIG. 6. *Cathode-ray oscillograms showing currents in special spark plug. Top trace, without suppressor. Bottom trace, with suppressor.* FIG. 7. *Spark plug current wave, taken with vibrator oscillograph.*



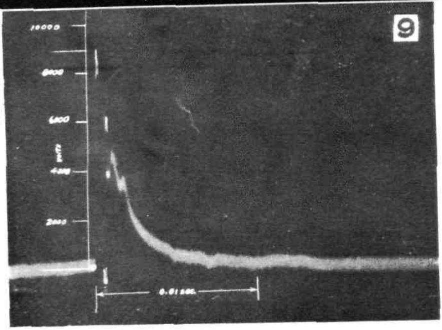
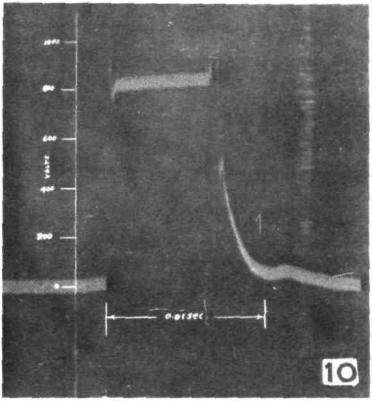
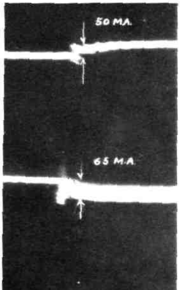


FIG. 8. *Secondary voltage on open circuit, taken with cathode-ray oscillograph.*
FIG. 9. *Secondary voltage on open circuit, taken with vibrator oscillograph.*





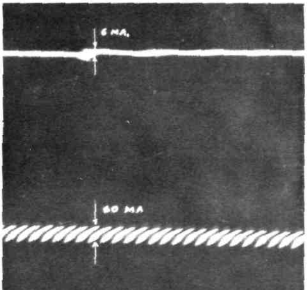


FIG. 10. *Voltage across spark plug during sparking, taken with vibrator oscillograph.* FIG. 11. *Top trace, current flowing in Oleo spark plug. Bottom trace, current flowing in AC spark plug. Both taken with cathode-ray oscillograph.* FIG. 12. *Cathode-ray oscillograms showing the action of a 50,000 ohm suppressor on spark oscillations. Top trace, current in AC plug with suppressor. Bottom trace, current in AC plug without suppressor.*

STUDIES ON THE USE OF FLAME SPECTRA IN CHEMICAL ANALYSIS[1]

By H. W. Lohse[2]

Abstract

Easily constructed accessory equipment for obtaining quantitative flame spectra has been designed, and is discussed. The causes of the variations in the results obtained by the flame spectrum method have been investigated and analyzed. Variations due to manipulation seem to be of little significance except perhaps as regards control of the acetylene pressure. Plate variations are significant and are the chief limiting factor in the accuracy of the method. A comparison of chemical and spectrographic analyses shows that the differences between the results obtained by these two methods are of the same order of magnitude as the differences obtained from plate to plate when the spectrographic method alone is used. Typical analytical data obtained by chemical methods and by the spectrographic method as well are presented.

Introduction

Inasmuch as a rapid and safe method for the estimation of some of the elements of the alkali and alkaline earth groups was required, investigations were made as to the suitability of the spectrographic method for this purpose. This study is based essentially upon the investigations carried out by Lundegårdh (14–18), who successfully developed a quantitative spectrographic method based upon flame spectra obtained by means of an air-acetylene flame.

The properties of flames and the nature of the problem dealt with in general in this paper have been thoroughly discussed elsewhere (2, 3, 4, 10, 24, 25). The general principles and technique of spectrographic methods have been discussed by several authors (1, 5, 8, 9, 12, 19). Specific details of the method of quantitative analysis by means of flame spectra have been given by Lundegårdh (14, 15).

The purpose of the present work was to study the accessory equipment used in quantitative analysis carried out by means of flame spectra, and to test the validity and utility of the data obtained. This paper deals with results obtained from an examination of about 1300 spectrograms, some of which were made for routine work. Such spectrograms are discussed only in so far as they are considered to be of significance as regards the above-mentioned purpose.

Apparatus

The Atomizer

In order to obtain a quietly burning flame, the liquid must be efficiently atomized without having too much air pass through the lamp. Gouy (10) used a glass atomizer, the design of which has had a significant effect upon

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Contribution from the MacLennan Laboratory of Physics, University of Toronto, Toronto, Canada, and the Department of Chemistry, Ontario Agricultural College, Guelph, Ontario, Canada. Some of the results presented here are taken from a thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy at the University of Toronto.
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the further development of glass atomizers as undertaken in this study (13), which has involved experimentation with about a score of different types of atomizers. Lundegårdh (15) uses atomizers of hard rubber, silver, gold and platinum. However, no details of design are given by this author. Two of Lundegårdh's silver atomizers have been used in the present investigation.

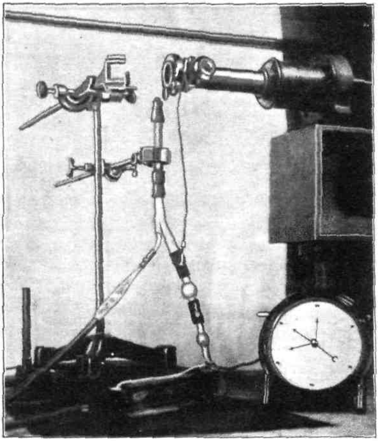

FIG. 1. *Arrangement of vessel and lamp.*

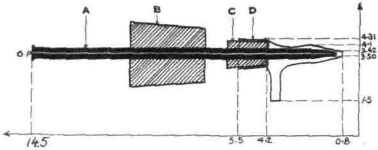

FIG. 2. *Glass atomizer (measurements in centimetres). A, glass capillary; B, C, rubber stoppers; D, cement layer.*

The theoretical aspects of atomization have been discussed by Schweitzer (7), Holroyd (11) and Castleman (6). According to Schweitzer, the atomization of a jet of liquid is most efficient when the jet emerges from the orifice in a turbulent state and when the break-up distance* of the jet is short. Holroyd deduces, on theoretical grounds, that the size of the droplets produced by atomization of a jet of liquid is inversely proportional to the mean angular velocity of the jet, this in turn being inversely proportional to the diameter of the orifice.** On the basis of the investigations referred to above, several types of glass atomizers were studied, and that shown in Fig. 2 was found to fulfil the requirements for atomization under the conditions prevailing in flame spectroscopy.

**The flow of a jet of liquid is said to be turbulent when the paths of the particles of liquid cross one another in a more or less disorderly manner, the particles having various transverse components of velocity. By the "break-up distance" is meant the distance from the nozzle at which the jet breaks up into a cone-shaped spray.*

***Holroyd derives the following equations which express better than words the significant factors which must be taken into consideration in studying atomization for the purpose of developing atomizers. Mean angular velocity* $= \omega = \frac{v}{d}\Psi\left(\frac{\rho v d}{\mu}\right)$, *where* v = *velocity of flow;* d = *diameter of orifice;* μ = *coefficient of viscosity;* ρ = *density of the liquid;* Ψ = *some function of* d, v, μ *and* ρ. *Mean diameter of drops* $= D = \sqrt[3]{k.\lambda/\rho\omega^2}$, *where* λ = *surface tension of liquid and* k *is a constant.*

This atomizer is made entirely of glass. By using a glass capillary as an air inlet, the flow of air is readily regulated in such a way that a fine jet of air of high velocity is produced in the nozzle of the atomizer. This jet acts partly as a jet pump and partly as a means of bringing the liquid into a state of violent turbulence in the nozzle. When the capillary is in the correct position, the jet of liquid should break into spray immediately on leaving the orifice, *i.e.*, the break-up distance should be zero. This position of the capillary is easily found by a little experimentation.* Various degrees of efficiency of atomization are obtained by changing the diameter of the orifice at the end of the tapered capillary and the end of the nozzle of the atomizer tube. The dimensions of the openings of the atomizer shown in Fig. 2 are suitable for lowest to medium efficiency (air pressures from 25 to 90 cm. of mercury.) If very high efficiency of atomization is required, a high air pressure (5–6 atm. or more) is necessary, the capillary being drawn out so that the pointed end is of very fine bore (0.2 mm. or less). By noting the appearance of the flame, the regulation is readily accomplished. The velocity of flow of the air–acetylene-vapor mixture can be varied over a wide range by varying the diameter of the orifice of the quartz tip of the lamp (Fig. 5). The stopper used to hold the capillary in the correct position in the tube is covered with deKhotinsky cement. Care should be taken to see that the capillary is rigidly fixed, and also that the connection between the stopper and the capillary is tightly sealed with the cement, so that no pin holes or crevices (which will retain residues of the liquid atomized) are left. Data obtained in an experiment with two different atomizers and atomizer vessels are shown in Table I.

TABLE I

ATOMIZATION OF DISTILLED WATER**

Atomizer vessel	Atomizer	Gm. of water passing through atomizer in 60 sec.	Mg. of vapor collected in the $CaCl_2$ tube
A (See Fig. 3)	Glass (See Fig. 2)	52.4	141
B (See Fig. 4)	Glass (See Fig. 2)	52.4	129
A	Silver† (from Lundegårdh)	135.2	98
B	Silver (from Lundegårdh)	135.2	96

***Air press., 87 cm. of mercury; temp., 20° C.*
†*This silver atomizer is furnished with an adjustable nozzle and is of medium efficiency (18).*

Lundegårdh reports that, under his earlier experimental conditions, in 60 sec., 15 cc. of water passed through the atomizer used and 37.7 mg. of water vapor passed up into the flame. Klemperer, using an electrolytic atomizer, delivered only 20 mg. to the flame in 60 sec. It should be mentioned that Lundegårdh now uses a platinum atomizer with narrow channels for air and liquid and a high air pressure (6 atm.), whereby very high efficiency of

**The fitting of the capillary in this position is readily accomplished by grinding the point of the capillary on a grindstone.*

atomization is obtained (18). The lower efficiency of the silver atomizer was due, no doubt, to the fact that the flow of liquid was not fully turbulent, and to the fact that this atomizer possessed an appreciable break-up distance.

The Atomizer Vessel

Designs for atomizer vessels are shown in Figs. 3 and 4. The small vessel (Fig. 3) is the most suitable and efficient one when an atomizer with no break-up distance and a short spray is employed, and it should be used when the atomizer shown in Fig. 2 is employed. In addition, it has the further advantage that it is exceedingly convenient to manipulate and is relatively easy to make. When atomizers with an appreciable break-up distance, or a long spray, or both, are used, a larger vessel such as that shown in Fig. 4, or the Lundegårdh vessel, may be employed. This is particularly advisable if a very high air pressure is applied. In Fig. 1 it will be seen that the connection between the vessel and the lamp is made by means of rubber tubing. If this tubing be of a *soft* quality, and of such dimensions that it can be connected to the vessel and lamp without any strain, the connection between these two can be very conveniently made or broken. The larger of the two rubber stoppers (see Fig. 2) used for the support of the atomizer, and for closing the vessel, should also be of soft rubber and of such a size that it fits into the opening in the vessel without any strain. Before use the vessel should be thoroughly cleaned out by means of a cleaning solution of concentrated sulphuric acid and potassium dichromate.

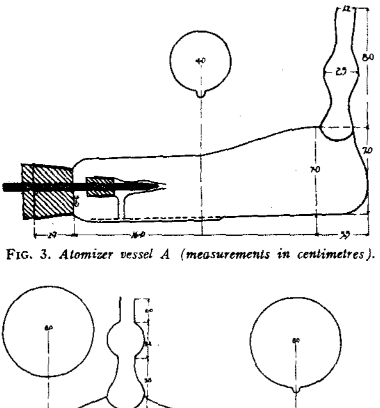

FIG. 3. *Atomizer vessel A (measurements in centimetres).*

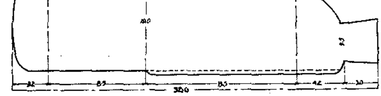

FIG. 4. *Atomizer vessel B (measurements in centimetres).*

The Lamp

The lamp shown in Fig. 5 is so designed that very little condensation of vapor takes place, even during long exposures. It is also very easy to clean. After completion of a series of analyses, the lamp is cleaned by running a

fine jet of water down through the tip, making sure that the sides of the lamp are rinsed all round. The last drops of water adhering to the sides are removed by blowing air through the lamp by means of a piece of rubber tubing held *very lightly* against the tip. In order to facilitate this removal, the individual glass parts of the lamp must occasionally be cleaned with cleaning solution. Since the air enters the lamp through the vessel only, the flame should always be started and extinguished while an appreciable current of air is flowing. A stopcock is placed between the acetylene valve and the manometer. When shutting off the gas, this stopcock is used, the acetylene valve remaining in the position that it occupied during the preceding exposure. In this way, on starting work again, a readjustment of the acetylene pressure to the previous value is very readily obtained. Should the flame accidentally strike back into the lamp, it need cause no concern as experience has shown that it dies down in the narrow orifice of the acetylene inlet. For further security a glass tube filled with absorbent cotton (see Figs. 1 and 5) is placed just in front on the acetylene inlet to the lamp. The bulb between the atomizer vessel and the lamp (Fig. 5, *b*) serves two purposes: *viz.*, (i) it acts as a drop catcher; and (ii) it serves to damp the rhythmic changes in air pressure produced by the atomizer, so that the gas mixture leaves the orifice of the lamp at a relatively constant pressure, thus producing a constant and quietly burning flame. With a properly adjusted set-up, these rhythmic changes should be practically eliminated.

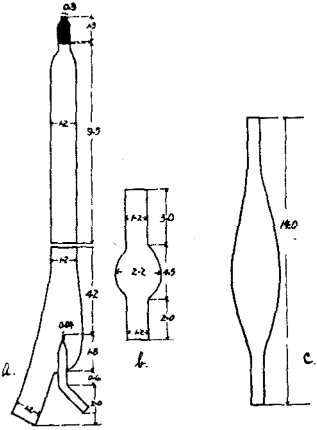

FIG. 5. *Lamp and accessory parts (measurements in centimetres).*

Exposure and Development of the Plates

In this series of experiments, the time of exposure was regulated by means of an iris diaphragm in which the celluloid segments were replaced by thin metal ones, and the mechanism was altered in such a way that exposures of any length of time could be made. With a little experience it is quite possible in this way to stop the exposure in a small fraction of a second. Throughout the experiments, a Hilger spectrograph *E2* with an optical train of quartz was used. With a slit about 0.1 mm. wide it was found that the residual lines due to potassium (4047.2 Å) and manganese (4030.8 Å) were re-

solved.* As these two elements frequently appeared together in the solutions analyzed, this slit width has been used throughout. Owing to the special nature of most of the spectrographic analyses carried out, panchromatic plates (Ilford Soft Gradation Panchromatic, 5 × 25 cm.) have been used. Both metol-hydroquinone and Rodinal (1 : 20, without potassium bromide) were used as developers and both were suitable. The development of these panchromatic plates has been described by Twyman (23). The line intensities were measured on a Moll microphotometer, Type A, adjusted in such a way that deflections over a range of 500 mm. were obtained.**

In developing the designs for the equipment mentioned above it has by no means been the intention of the writer to disregard the accessory equipment designed by Lundegårdh (14) which serves the purpose very well. The spectrographic method possesses such advantages that it can profitably be employed generally, and it is hoped that access to designs for accessory equipment such as can be made in any analytical laboratory will facilitate its employment.

Experimental

The spectrographic method of analysis, in common with all methods of measurement, is burdened with sources of variation† which limit the accuracy of the method. Generally speaking, the variations in the results obtained by the spectrographic method are due partly to variations in *(a)* the air pressure, *(b)* the acetylene pressure and *(c)* the time of exposure, and partly to "plate variation"‡ *viz.*, *(i)* uneven transparency of the plate itself, and *(ii)* uneven development of the plate. An analysis of these variations based on experimental data is shown in Tables II to VIII. It should be added that all solutions used in these experiments were kept in paraffined bottles. In this way the possibility of contamination of the solutions by any substance dissolved from the glass of the containers is obviated.

Experimental Conditions Influencing the Line Intensity

The Effect of Different Air Pressures

It is seen in Table II that the variation of the line intensity for a change of 1 cm. of mercury in the air pressure amounts to about 1% for the sensitive Na-line, and to about 0.3 to 0.4% for the less sensitive K-line. The intensity of the blackening of the band varies much less. By comparing the relative intensities of the corrected and uncorrected lines, a good indication of the significance on the plate variation is obtained (see also the following series). It is possible to control the air pressure by means of a suitable pressure valve to within 0.1 to 0.2 mm. of mercury. In this way, variations due to changes in air pressure are reduced to a negligible amount.

** The large dispersion of this spectrograph should be noted when comparing the results presented here with those obtained by using spectrographs of lower dispersion.*

*** With this arrangement, the reproduction of the readings was very satisfactory.*

†The term variation is used throughout in this paper instead of the term error, because only systematic variations lead to actual errors.

‡Lundegårdh assumes (15) that the chief cause for the variation in the data obtained by his spectrographic method is due to "plate errors", viz., variation due to heterogeneity of the developed photographic plate itself. This variation is in this paper referred to as "plate variation."

TABLE II

THE EFFECT OF DIFFERENT AIR PRESSURES UPON THE LINE INTENSITY

Air pressure, cm. Hg.	Relative intensity					
	Na-line (5890.2 Å, 5896.2 Å), 0.006 mg. Na per cc.					
	Experiment I			*Experiment II*		
	Band	Na-line uncorrected	Na-line corrected†	Band	Na-line uncorrected	Na-line corrected
70	100	100	100	100	100	100
80	97	85	91	98	87	92
90	97	79	82	96	77	80
	K-line (4047.2 Å), 0.782 mg. K per cc.					
	Experiment I			Experiment II		
	Band	K-line uncorrected	K-line corrected	Band	K-line uncorrected	K-line corrected
70	100	100	100	100	100	100
80	96	94	98	100	97	97
90	93	87	92	99	93	94

† *The correction for plate variation is carried out according to Lundegårdh (15), using the equation* $I = I_1 . n/n_1$ *(I = corrected line intensity,* I_1 *= uncorrected line intensity,* n_1 *= band reading, n = average of all bands.*

NOTE.—*Exposure, 1 min.; acetylene pressure, 34 cm. of water.*

The Effect of Time of Exposure

Considering the fact that the shutter used takes only a fraction of a second to open or close, and thus delimits the exposure very sharply, it is seen from Table III that the effect of the small variations present is negligible. By increasing the time of exposure, considerable increase in the line intensity is obtained, especially for the most sensitive line (calcium).

TABLE III

EFFECT OF TIME OF EXPOSURE ON THE RELATIVE LINE INTENSITY

Time of exposure, min.	Relative line intensities								
	Ca-line (4226.7 Å); *0.0401 mg. Ca per cc.*			K-line (4047.2 Å)*; 0.1955 mg. K *per cc.*			Ba-line (5535.5 Å); 0.328 mg. Ba per cc.**		
	Band	Ca-line uncorrected	Ca-line corrected	Band	K-line uncorrected	K-line corrected	Band	Ba-line uncorrected	Ba-line corrected
1	100	100	100	100	100	100	—	—	—
3	111	90	84	98	90	92	100	100	100
5	113	80	73	93	79	85	93	98	96
10	108	62	59	92	71	77	94	101	93

* *The potassium lines were produced under conditions which differed somewhat from those under which the other two lines were produced.*

** *The plate used was rather old.*

NOTE.—*Air press., 88 cm. of mercury; acetylene press., 34 cm. of water.*

The Effect of Different Acetylene Pressures

The standard deviation of a single flame band is computed by the formula $m = \pm \sqrt{\Sigma d^2/n - 1}$, where n is the number of bands and d the deviation from the mean. The dispersion about the mean is computed by the formula $M = \pm \sqrt{\Sigma d^2/n(n - 1)}$, where n and d have the same meanings as above. These numerical dispersions are finally computed in percentages of the mean of all the replications. These two measures of variation are also used in Tables V, VI and VII.

Although the data in Table IV are based upon experiments in which there existed a considerable plate variation, nevertheless there is a sufficiently high correlation between acetylene pressure and line intensity to make it evident that a variation of 1 cm. of water in the acetylene pressure may produce a variation of several per cent in the line intensity of an element whose residual line is very sensitive (calcium, sodium). This is not in agreement

TABLE IV

THE EFFECT OF DIFFERENT ACETYLENE PRESSURES ON THE RELATIVE LINE INTENSITY

Acetylene pressure, cm. water	Relative line intensities					
	Na-line (5890.2 Å; 5896.2 Å); 0.0006 mg. Na per cc.					
	Experiment I			Experiment II		
	Band	Na-line, uncorrected	Na-line, corrected	Band	Na-line, uncorrected	Na-line, corrected
35	100	100	100	100	100	100
34	101	101	100	100	94	99
33	100	97	97	98	100	96
32	99	97	98	99	99	98
	Ca-line (4226.7 Å); 0.0401 mg. Ca per cc.					
	Experiment I			Experiment II		
	Band	Ca-line, uncorrected	Ca-line, corrected	Band	Ca-line, uncorrected	Ca-line, corrected
35	100	100	100	100	100	100
34	107	104	102	91	90	100
33	105	102	103	89	87	102
32	99	95	105	86	83	103
	K-line (4047.2 A); 0.782 mg. K per cc.					
	Experiment I			Experiment II		
	Band	K-line, uncorrected	K-line, corrected	Band	K-line, uncorrected	K-line, corrected
35	100	100	100	100	100	100
34	101	100	99	98	97	99
33	101	99	98	91	86	100
32	99	98	99	94	95	101

NOTE.—*Air press., 9 cm. of mercury; time of exposure, 1 min.; diameter of orifice of quartz tip of lamp, 3 mm.*

TABLE V

VARIABILITY IN THE FLAME BANDS ADJACENT TO THE LINES

—	Standard deviation of a single flame band, *m*, %		Average deviation about the mean, of all flame bands, *M*, %	
	Experiment I	Experiment II	Experiment I	Experiment II
Na-line	0.8	1.3	0.4	0.7
Ca-line	3.8	9.7	1.9	4.0
K-line	1.0	2.5	0.5	1.4

with Lundegårdh's conclusion (15) that a deviation of 1 cm. of water in the acetylene pressure is not significant.

Variability in the Blackening of the Flame Bands

The plates referred to in Table VI are arranged according to the average galvanometer deflection due to the bands. This arrangement should give a

TABLE VI

VARIABILITY IN THE BLACKENING OF THE FLAME BANDS

Plate No.	Number of spectrograms, *n*	*m*, %	*M*, %	Mean galvanometer deflection, mm.	Spectrograms
61	7	0.7	0.3	483	Soil extracts and KCl standards.
88	6	4.3	2.2	478	Soil extracts and KCl standards.
58	5	3.4	1.6	444	KCl solutions.
69	5	0.2	0.07	425	KCl solutions
68	9	2.9	1.0	372	Soil extracts and KCl standards
70	5	1.4	0.6	360	Soil extracts and KCl standards
90	9	3.9	1.3	343	Feldspar (solution) and KCl standards
66	9	4.0	1.4	287	Soil extracts, phlogopite (solution) and KCl standards
89	9	3.6	0.4	261	Soil extracts, feldspar (solution) and KCl standards
65	9	2.3	0.8	250	Soil extract and KCl standards.

fair indication of the effect of the degree of development on the plate variation. This series of experiments indicates (Table VI) that a certain amount of fogging of the plates does not influence the accuracy of measurement of the line intensities when the light source used is powerful enough to provide for sufficiently large deflections. This is in agreement with the viewpoints of Ornstein, Moll and Burger (19), whereas Lundegårdh (15) considers fogging a significant cause of plate variation.

Variability of the Line Intensity when Different Numbers of Replications were made

The replications shown in Table VII were made in consecutive order across the plate, and the line readings were corrected as described in the footnote to Table II.

TABLE VII

VARIABILITY IN LINE INTENSITY WITH NUMBER OF REPLICATIONS

No. of replications	Na-lines, (5890.2 Å; 5896.2 Å) 0.0006 mg. Na per cc.		Ca-line, (4226.7 Å) 0.0401 mg. Ca per cc.		K-line, (4047.2 Å) 0.782 mg. K per cc.	
	m, %	*M*, %	*m*, %	*M*, %	*m*, %	*M*, %
2	1.6	1.1	3.0	2.1	0.69	0.49
3	0.55	0.00	2.3	1.1	0.56	0.32
4	1.2	0.62	0.76	0.40	1.4	0.70
5	1.9	0.82	1.8	0.79	1.9	0.83
6	2.4	1.0	1.6	0.66	2.4	1.0
7	3.1	1.2	1.5	0.56	2.8	1.1
8	3.5	1.2	1.3	0.15	3.0	1.1
9	3.0	1.7	—	—	3.0	1.7

NOTE.—*Air press., 88 cm. of mercury; acetylene press., 34 cm. of water; exposure, 1 min.; orifice of lamp, 3 mm.*

The variability (for all the replications) in the flame bands adjacent to the lines was as shown in Table VIII.

TABLE VIII

VARIABILITY IN THE FLAME BANDS ADJACENT TO THE LINES (COMPUTED ON A BASIS OF ALL THE REPLICATIONS)

Na-line		Ca-line		K-line	
m, %	*M*, %	*m*, %	*M*, %	*m*, %	*M*, %
5.3	1.9	4.2	1.5	7.3	2.0

That we are dealing here with systematic variation appears from the fact that the dispersion about the mean increases with the number of replications. The dispersions about the mean of the first successive either two or three spectrograms of the calcium line (4226.7 Å) are very high, owing to the fact that the intensity of the line on the second spectrogram deviated greatly. The galvanometer deflections due to the first three replications of the calcium line were 334, 320 and 332, while those due to the last three replications of the calcium line were 332, 332 and 330. In the latter case, both *m* and *M* are negligible. However it does at times (though not often) happen in general routine work, that a line deviates considerably.

Computation of Results from Experimental Data

As the plate variation is systematic and causes actual errors, the results obtained by the spectrographic method discussed in this paper should not be computed on the basis of a theoretical standard curve, but rather on the basis of an empirical one. To summarize, the rules to be followed in order

to obtain the most reliable and accurate results are as follows: (i) the results should be computed on the basis of an *empirical* standard curve*; (ii) standards and unknown solutions should be of approximately the same concentration; (iii) the range of concentration used in a series of analyses on the same plate should never be too wide; (iv) the replications should be eventually made on different plates. Even though the plate variation may be different for different types of plates, the above-mentioned rules are probably capable of quite general application. General rules for minimizing the plate variation are not readily given. It seems that slow development and thorough shaking of the developer and fixer (during the first few minutes at least) result in the most uniform development. With panchromatic plates such as those employed here, care should be taken not to use plates that have been stored too long (23).

(For further discussion of the problem dealt with in this paragraph see References (5) and (15).)

Quantitative Analysis

Comparative chemical and spectrographic analyses were carried out intensively by Lundegårdh (14, 15), and, on the average, satisfactory results were obtained. Such comparative analyses have also been carried out during the course of the present studies, the results of which are shown in Table IX.

TABLE IX

Comparative chemical and spectrographic analyses

Sample	Spectrographic method	Chemical methods	Nature of chemical method	Difference between the two methods, %
1. Orthoclase	Total, 8.56% K	8.54% K 8.87%	Colorimetric Using K_2PtCl_6	0.23 3.60
2. Muscovite	Total, 7.89% K	8.00% K	Using K_2PtCl_6	1.4
3. Feldspar	Total, 9.34% K	9.30% K	Colorimetric	0.43
4. Soil extract	Extracted 480 mg. K per kg. of soil	Extracted 488 mg. K per kg. of soil	Colorimetric	1.70
5. Ba in paper	65.5 mg. Ba in 77.78 gm. of paper	67.4 mg. Ba in 77.78 gm. of paper	Gravimetric	2.90

It is interesting to note that the differences between the results obtained by these two methods are of the same order of magnitude as the band and plate variation discussed above.

**When the standard curve is drawn an appreciable plate variation is readily recognized if present.*

Chemical Analytical Procedures

A suspension of the finely ground potash-bearing mineral (Nos. 1, 2 and 3) in concentrated sulphuric acid, to which hydrofluoric acid was added, was evaporated to dryness. This was repeated until all the silica was removed. Hydrochloric acid was added and the suspension evaporated to complete dryness. This operation was repeated twice. The residue was then taken up with hot dilute hydrochloric acid and filtered through ashless filter paper. The filter paper with the residue (which was very small in all cases) was ashed at as low a temperature as possible, the ash taken up with hot dilute hydrochloric acid, filtered off, and this filtrate added to the main portion of the filtrate. The final residue was almost negligible; it was not fused with sodium carbonate, in order that alkalies might not be introduced. On the basis of experience obtained in other work, it is felt that such a fusion would not, in this case, have influenced the data significantly. The combined filtrates were evaporated to complete dryness, the residues taken up with water and diluted to a volume suitable for the spectrographic and colorimetric measurements which were carried out directly on this solution. By the colorimetric procedure, the potassium was precipitated directly from these solutions by means of sodium cobaltinitrite, prepared according to Biilmann (13), and the potassium was estimated indirectly by determining the cobalt in the precipitate (13). The good agreement between the spectrographic and colorimetric methods may be ascribed to the fact that in both cases the results were obtained by using a fully standardized technique, and by basing the final computation of the results on an empirical standard curve. The same standard solutions were used in both cases. In the two cases where potassium chloroplatinate has been used, the alkali chlorides were very carefully isolated, although no attempt was made to correct for the possible presence of small amounts of caesium and rubidium. This may explain the somewhat higher result obtained when potassium chloroplatinate was used as a precipitating agent.

The soil (No. 4) was extracted with water. To a suitable aliquot of the extract was added a few drops of dilute sulphuric acid, and the solution was evaporated to dryness. Organic matter was burned away at low heat, the residue taken up with water, filtered off, and the filtrate concentrated to a volume suitable for spectrographic and colorimetric analysis.

In the case of the determination of barium* in paper, 77.78 gm. of paper was ashed and a suspension of the ash in concentrated sulphuric acid and in hydrofluoric acid was evaporated to dryness several times to remove silica. The residue was taken up with hydrochloric acid in order to dissolve alumina and other soluble bases. The insoluble residue (mostly barium sulphate) was filtered off and ignited (weight of ash, 0.1639 gm.). This residue was reduced with cane-sugar charcoal and the last traces of insoluble material were fused with sodium carbonate. The hydrochloric acid solution of the melt

**This study was undertaken at the request of the Ontario Research Foundation, Toronto. The chemical work was done by Mr. O. J. Schierholtz, Research Chemist of the Research Foundation, to whom the author is indebted for permission to publish these results.*

was evaporated to dryness twice and the volume finally made up to 50 cc. The barium content of this solution was determined spectrographically and by the chemical gravimetric method. It should be mentioned that the acid blank and the acid filtrate containing the soluble bases did not contain any barium (spectrographic test).

The significance of this comparison of the two methods lies in the fact that these examples all represent cases where only very tedious chemical methods lead to reliable results. Also it shows that the spectrographic method serves the purpose of macroanalysis as well as microanalysis.

Acknowledgments

Acknowledgments are due to Prof. R. Harcourt, Head of the Department of Chemistry, Ontario Agricultural College, for suggesting these studies; to Prof. E. F. Burton, Director of the MacLennan Laboratory of Physics, University of Toronto, for providing facilities for the investigations; to Prof. H. J. C. Ireton and Dr. Elizabeth Allin of the same institution for valuable advice; and to Mr. R. H. Chappel, glass blower, also of the MacLennan Laboratory, who carried out the glass blowing on the apparatus designed by the author. It might be added that much more equipment has been experimented with than has been discussed in this paper. Finally the author wishes to thank Prof. H. Lundegårdh, Stockholm, Sweden, for much valuable advice and for furnishing him with two silver atomizers. The author wishes also to express his gratitude to the Canada and Dominion Sugar Company, Chatham, Ontario, for financial support in this work.

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MICROSCOPIC FEATURES OF THE PHALEN SEAM, SYDNEY COAL FIELD, N.S.[1]

By W. R. Newman[2]

Abstract

The microscopic and chemical nature of the anthraxylon, attritus and fusain of the Phalen coal were studied. The attritus is composed of humain (humic degradation matter), opaque matter, resins, waxes, cuticles and spores. Pyrite and other mineral matter also were detected. The microstructural features of the coals of the Phalen seam are so distinctive that they can be divided into three groups. Coal of Group 1 is characterized by a high spore, low resin, and low pyrite content, and was formed under open-water conditions which existed in the central part of the swamp. Group 3 coal has a low spore, high resin, and high pyrite content, and originated under conditions which prevailed near the shore line of the original swamp. Group 2 is a transition stage between Groups 1 and 3. The microstructure of the coal indicates that future mining possibilities are most favorable in the vicinity of the Bridgeport anticline.

The sulphur in the Phalen seam is due mainly to pyrite and is irregularly distributed throughout the seam. The pyrite was probably formed from iron- and sulphur-bearing solutions which entered the swamp in the vicinity of Cape Percy. The iron and sulphur were precipitated by the organic products of decay, producing a disseminated pyrite in the coal.

Nine types of megaspores and eleven types of microspores were determined on the basis of their morphological appearance. Correlation by means of these spores was not possible, however, owing to the variability in the spore content and the lack of any persistent distinctive spore.

Introduction

The land portion of the Sydney coal field is only the fringe of an extensive coal basin that extends far out beneath the sea. The future of the field depends upon the undersea development of the collieries. In 1928 the Geological Survey of Canada and the Fuel Division of the Mines Branch undertook a joint survey of the Phalen seam, the largest and most important seam in the field. Thirteen locations in the Phalen seam, some inland and some under the sea, were carefully selected, and complete pillars varying from 5.5 to 8.5 ft. in height and 1 ft. square were extracted. These pillars were examined as closely as possible before removal, and were subdivided into 20-24 sections on the basis of their macroscopic appearance. The pillars were carefully packed and shipped to Ottawa where microscopic and chemical examinations of the sections were made.

The writer, under the supervision of Dr. B. R. MacKay of the Geological Survey of Canada, undertook the microscopic investigation of the pillars. The purpose of this study was to determine the nature and constitution of the coal in the Phalen seam, the varying character of the constituents of the coal throughout the field, and the physiographic conditions under which the

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Published with the permission of the Director, Geological Survey of Canada, Ottawa. The investigation was part of a more extensive study of the coal deposits of the Sydney coal basin, N.S., and of the physical constitution of the coals of Canada. This paper is a synopsis of a thesis presented as part of the requirements for the degree of Doctor of Philosophy at the University of Toronto. The investigation was made for the Geological Survey of Canada which supported it financially and provided materials and laboratory facilities.

[2] *Postgraduate student, University of Toronto, and student assistant, Geological Survey of Canada.*

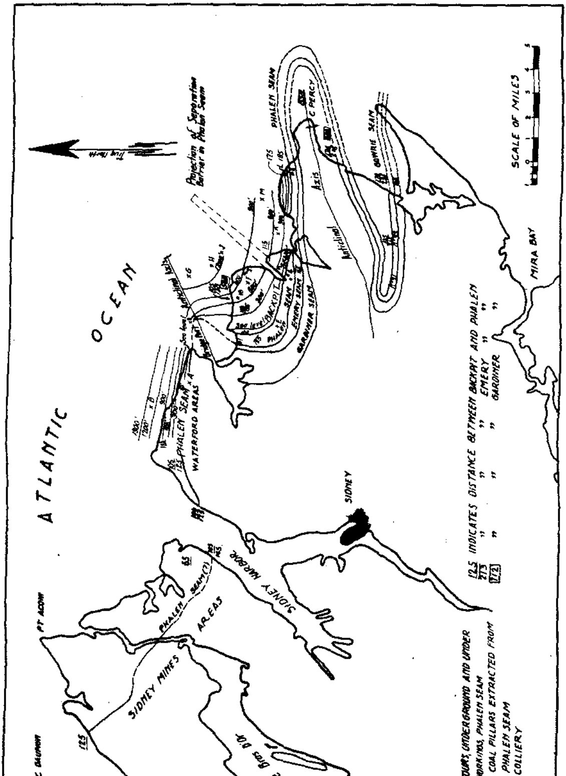


FIG. 1. *Map showing the possible extent of the Phalen seam and its underground and undersea development.*

coal was laid down. In addition, the nature and distribution of the sulphur in the coal, and the possibilities of correlation by means of the spore content of the seam were investigated.

Phalen Coal Seam

The Phalen seam supplies more coal than any other seam in Canada. Stratigraphically, it is part of the *Anthracomya* zone of the Morien series (6) and occurs approximately 150 ft. above the base of the zone. The seam is believed to continue throughout the whole extent of the Sydney field (Fig. 1).

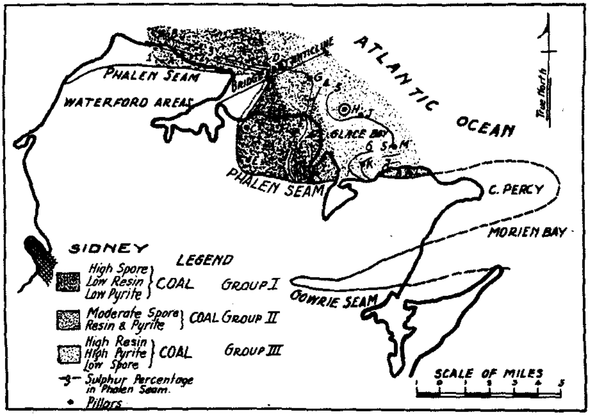


FIG. 2. *Map showing variation in microstructure and sulphur distribution of the Phalen coal seam.*

It follows the structural features of the field, but has been separated by folds into a series of apparently separate seams. The structural features of the Phalen seam are relatively simple. In the Morien basin, it forms the two limbs of a syncline. It has not been traced around the Cape Percy anticline, but it reappears in the Glace Bay region where it dips gently seaward. The Bridgeport anticline is the locale of the best coal in the Phalen seam. In the Lingan basin, the seam dips rather deeply seaward (Fig. 2). The extension of the seam under Sydney Harbor and on the Sydney Mines side has not been proved. The folding of the seam as a whole appears to become less intense seaward.

Macroscopic Description of Phalen Coal

The coal of the Phalen seam is a banded bituminous steam coal, possessing fair coking properties. It has a high sulphur and ash content which varies

in different parts of the seam. In the hand specimen shown in Plate I, Fig. 1, the three normal banded components of bituminous coal are recognized, —anthraxylon or bright coal, attritus or dull coal, and fusain.

The anthraxylon appears as bright black bands with a vitreous lustre. These bands are never more than an inch in thickness and are of a lenticular nature, pinching out laterally. They are uniformly distributed from the top to the bottom of the seam. There is, however, an apparent increase in the proportion of anthraxylon in the eastern portion of the field; it is undoubtedly purer than the other components of the coal, and is relatively free from mineral matter, which occurs mainly in the attritus.

The attritus is the groundmass in which the anthraxylon is embedded, and has a characteristic dull lustre. In the hand specimen it forms the greater bulk of the coal and is varied in structure. In the dull coal, numerous microscopic lenticles of anthraxylon are noticeable. In addition, the dull coal contains the largest proportion of mineral impurities; but mineral matter which is intimately associated with the attritus is not visible macroscopically.

The fusain is a dull, powdery, charcoal-like substance, finely distributed throughout the coal. Considering the fragile nature of the substance, it is strange that delicate fern and leaf impressions in the fusain have been preserved. These structures are especially noticeable in the hand specimen where the fusain is parallel to the bedding. The fusain occurs in thin lenticles about $\frac{1}{8}$ in. or less in thickness. The fusain is more abundant at the top and the bottom of the pillars, and increases greatly in proportion in the eastern part of the field. In most cases, fusain does not appear to contain mineral matter.

The Phalen coal varies from an anthraxylous-attrital to an attrital-anthraxylous type (3, pp. 22-25). The banded structure may be regarded as an alternation of a coarsely and a finely banded type. As the groundmass of the coal is translucent in thin section, the Phalen coal is a normal bituminous or bright coal.

Microscopic Character of the Phalen Seam

Examination of thin sections of coal by transmitted light was the chief method of study used in this investigation. Thiessen's method (3, pp. 22-25) with a few modifications was the one adopted.

The microscopic components of the Phalen coal may be classed under three main headings: anthraxylon, attritus and fusain.

Anthraxylon

Anthraxylon is present throughout the whole seam and occurs in lenticular bodies. Under the microscope, in thin section, it is usually of uniform mahogany color giving little indication of structure (Plate 1, Fig. 2). Cell structure in the anthraxylon was found only in a few cases. Normal, faint cell structure can occasionally be seen. The anthraxylon has been practically

all derived from ordinary woody tissue. It is believed that anthraxylon is composed chiefly of lignin, the cellulose having been largely decomposed. The structureless character of the anthraxylon would indicate that biochemical action had been active in the transformation of cellular plant tissue into the homogeneous anthraxylon. The plant tissue may have been infiltrated by colloidal substances and later transformed into the anthraxylon lenticle.

The coking properties of coal are due mainly to the anthraxylon components. A coal with a high percentage of attrital matter is usually a poorer coking coal than one of an anthraxylous nature. Not all anthraxylous coals will coke, as this property is possessed only by coals in certain stages of coalification.

Attritus

Attritus forms the most interesting and complex part of coal. In its broadest sense, it includes not only all comminuted plant matter contributed from the original swamp, but it may also contain microscopic lenticles of anthraxylon and small particles of fusain. The attritus of the Phalen coal consists of the following constituents: humic degradation matter, spore exines, resins, cuticles and waxes, opaque matter and mineral matter.

Humic Degradation Matter

The term "humic degradation matter" is rather difficult to define, and, as it is an unwieldy expression, the writer here proposes to adopt the term "humain" as synonymous with it. Humain is composed of the fragments of the cell walls of all sorts of plant and woody tissue and the contents of cells of plants (Plate III, Fig. 1). These have been greatly altered by biochemical decay and deposited in the swamp. It generally forms the bulk of the attritus.

Spore Exines

Spores are the reproductive bodies of the lower plants. As yet, not enough is known about spores found in coal to classify them genetically. The classification adopted is strictly morphological and is based on the external appearance of the spores. The American investigators (8) maintain that the limiting sizes for microspores are 13–70 μ, and for megaspores, 500–3000 μ. The English coal microscopists (7, p. 8) find a range of 15–100 μ for microspores, and 130–2500 μ for megaspores. These figures however are subject to change. It must be realized that the size of a spore does not distinguish a microspore from a megaspore as much as do its mode of development and function as a spore in the living plant. The microspores develop in large numbers in a microsporangium (Plate II, Fig. 2), but only one or a few megaspores develop in a megasporangium.

The spores are spherical bodies, but owing to putrefaction the living matter enclosed by the shell, or exine, is lost. The spore on being buried is flattened by pressure. As the exines are extremely resistant to decay, they may become concentrated in the coal when conditions are favorable. In the horizontal sections, the spores are more or less circular, and the tetrad markings may be frequently distinguished. When examined in vertical sections, they appear

as collapsed rings, the living matter within the spore having been decomposed and the spore flattened by pressure. Microspores are by far the more numerous of the two spore types. The megaspores, however, on account of their larger size, are more easily studied (Figs. 3 and 4).

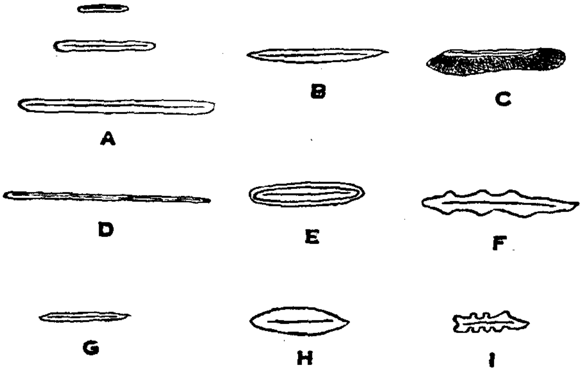


FIG. 3. *Megaspores found in the Phalen coal seam.* × *100.*

The walls of the spores are frequently ornamented and sculptured, and it is on the basis of these ornamentations that the spores are classified. Spores may be serrated or they may be perfectly smooth. They may have wings, spines, tubercles and tufts.

Cannel coals are high-spore coals and are the result of the accumulation of spores which were conveyed by the wind or currents to a swamp, lake, lagoon, or sea where tranquil open-water conditions prevailed. A high-spore coal would thus indicate open-water conditions of deposition. Spores are the important oil- and gas-yielding constituents in coal.

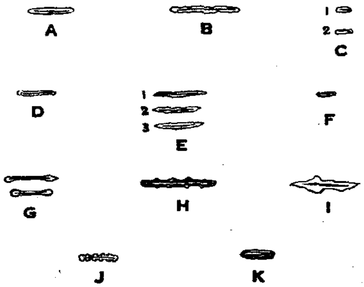


FIG. 4. *Microspores found in the Phalen coal seam.* × *630.*

A description of the microspores and megaspores found in the Phalen seam will be given later.

Resins (11)

Resins are conspicuous bodies in most coals. They are very resistant to putrefaction and to destructive agents of any sort. Resins are the remains of the matter stored up in resin ducts and certain cells of plants. On account of the appearance and mode of occurrence of oval-shaped, reddish-colored bodies in the higher-rank coals, it is assumed by analogy that these are the remains of the original resin in the plants. The abundance and distribution of the resins are an indication of the amount of decay undergone by the original plant material. Resins are usually amongst the last products to survive subaqueous biochemical decomposition. As a result of dehydration, progressive chemical reduction, and the collapse of the plant tissue due to pressure, the resins have been concentrated in coal so that their volume is greatly increased relative to that of the other constituents.

There are three modes of occurrence of resinous bodies in the Phalen seam.

1. *Flattened elongated bands in the anthraxylon.* The resinous bodies were compressed, flattened and elongated in woody tissue which was changed into anthraxylon. This represents the stage of least decay.

2. *Disseminated bodies throughout the attritus.* The resinous bodies begin to increase in proportion, under putrefactive conditions, and are found scattered throughout the attritus with the bodies more or less resistant to decay. This stage is intermediate between the first and third types and is the commonest.

3. *Aggregations of resinous bodies in the attritus.* When putrefaction has reached such a stage that the humic matter has disappeared and only the most resistant bodies remain, aggregations or clusters of resinous bodies are found. This is the stage of greatest decay (Plate II, Fig. 5).

The proportion of resinous bodies varies in different parts of the Phalen seam, thus indicating that conditions of decay were not uniform in different parts of the seam.

Cuticles and Waxes

Closely allied to the resins in nature are the cuticles, As is the case with the present day plants, the Carboniferous flora had certain of their tissues protected with a thin layer of cuticular matter. This waxy material is extremely resistant to putrefaction and, as a result, when plants accumulate in a swamp, it increases in proportion as the more readily decomposed tissues of plants decay. In nearly all coals, fragments of cuticular tissue are common. In thin section, they appear as lemon-yellow elongated bands, characterized by a serrated border (Plate III, Fig. 2). The borders result when the cuticles conform to the cells of the once underlying epidermal tissue.

Oval-shaped bodies of lemon-yellow color, which are believed to be of a waxy nature, are occasionally seen in the Phalen coal (Plate II, Fig. 6). They are similar in shape to the resins, but are not of the same color and do not hold the same relations to the attritus.

Opaque Matter

The nature of the opaque matter is not yet fully understood. It may have been derived from the cell contents of plants. Opaque matter, in places, appears to be a highly altered substance similar to fusain. This is the case in the Phalen seam where the opaque matter appears to be a finely divided variety of fusain. In addition, the opaque matter is most abundant in the Phalen seam where the plant material in the coal has undergone great decay (Plate III, Fig. 3).

Mineral Matter

The source of the mineral matter in coal may be extraneous or indigenous. It may be of organic or inorganic origin. In the Phalen seam, calcite has been found, and the mineral is stained by iron in certain parts of the seam. Quartz, carbonates and gypsum have been determined microscopically. Pyrite is the most important mineral of economic interest in the seam, and will be discussed more fully later. Shaly matter is common in portions of the seam and is chiefly of a carbonaceous nature.

Fusain

One of the most interesting constituents of coal is fusain. It includes the more highly carbonized matter consisting of thin layers and lenticles of original plant tissues whose structure may or may not be retained. Under the microscope, the fusain, when cellular, has the appearance of a finely reticulated structure. Some of the most delicate plant structures are sometimes exquisitely preserved in the fusain.

The fusain of the Phalen seam is of two types microscopically.

1. Fusain with well defined cell structure (Plate II, Fig. 4).
2. Fusain composed of structureless, compact masses probably formed by the collapse of the cellular structures (Plate III, Fig. 3).

A few cases of gradation from anthraxylon to cellular fusain were observed. The sequence begins with anthraxylon, which gradually becomes cellular, then more opaque, and finally cellular fusain is formed.

Fusain, if present in any quantity, is believed to be deleterious to coking, but this is debatable. It is also believed to furnish most of the dust found in coal mines. In the Phalen seam the fusain forms about 2% of the coal.

Analyses

Analyses of anthraxylon, attritus and fusain were furnished through the courtesy of R. E. Gilmore, Superintendent of the Fuel Research Laboratories of the Department of Mines at Ottawa. The different components were carefully selected from the pillars, special care being taken to keep the specimens as pure as possible.

The proximate analyses indicate that fixed carbon is highest in anthraxylon and lowest in attritus. As would be expected, owing to the presence of spores, resins and cuticles, the volatile matter is highest in attritus and lowest in

TABLE I

ANALYSES OF COMPONENTS OF PHALEN COAL

—	Anthraxylon		Attritus		Fusain	
	As received	Dry	As received	Dry	As received	Dry
Proximate analyses						
Moisture, %	2.7	—	2.1	—	1.3	—
Ash, %	4.1	4.2	7.2	7.3	17.2	17.4
Volatile matter, %	29.3	30.1	32.6	33.3	22.5	22.8
Fixed carbon, %	63.9	65.7	58.1	59.4	59.0	59.8
Ultimate analyses						
Carbon, %	80.0	82.3	76.1	77.8	69.7	70.7
Hydrogen, %	5.0	4.8	5.1	5.0	3.0	2.9
Ash, %	4.1	4.2	7.2	7.3	17.2	17.4
Sulphur, %	1.3	1.3	2.3	2.3	1.1	1.1
Nitrogen, %	1.4	1.4	1.5	1.6	0.5	0.5
Oxygen, %	8.2	6.0	7.8	6.0	8.5	7.4
Other data						
Calories per gm. gross	7,860	8,080	7,560	7,725	6,205	6,290
B.t.u.'s per lb. gross	14,150	14,550	13,610	13,910	11,170	11,320
Carbon-hydrogen ratio	16.0	17.0	14.9	15.7	23.1	24.3
Fuel ratio	2.20		1.80		2.60	
Softening temperature of ash, ° F.	2000		2115		2680	
Specific gravity (apparent)						
Lumps	1.27		1.42		1.48	
Fines	1.26		1.23		1.29	
Coking properties	Good		Fair		Non-coking	

fusain. Since the anthraxylon is the purest component in coal, it contains the least ash. The attritus with its multivariant components has a high ash content. The fusain however has the highest ash content. The cellular spaces of the fusain were probably impregnated with mineral matter which, strangely, is not pyritiferous. Carbonates and silica probably compose most of the fusain ash. The attritus contains the highest percentage of sulphur.

The coking properties of the various components are interesting. It has been suggested that coals composed for the most part of woody constituents are the best coking coals. Anthraxylon, the component richest in woody matter, has the best coking properties, while fusain does not form coke at all. Singularly, the heating value is highest in the anthraxylon and least in the fusain.

Physiographic Conditions of Deposition of the Phalen Seam

A knowledge of the conditions under which the Phalen seam was laid down is of importance to the coal miner and geologist, not only from a scientific point of view, but for the information it may furnish regarding the more

economical methods of selective mining, upon which the future of the fiel depends. The exhaustion of the land workings and the growing dependenc of the field on undersea mining has necessitated the adoption of more carefı methods of extracting the coal.

As far as the writer is aware, no attempt has yet been made to interpre the physiographic conditions of deposition of an individual coal seam on th basis of the microstructure of the coal. Any features revealed by the micro structure which would indicate the more favorable localities for future minin development would be of value to the coal operators.

Hayes and Bell (6) state that flood-plain deposition in a progressively sub siding river valley was the mode of origin of the Sydney coal field. Th coal-forming swamps were subject to different conditions and, as a result coal of various types and qualities was deposited. It is quite possible that throughout the whole coal field, streams were flowing into the swamps an contaminating the plant matter with various inorganic sediments. Condition near the border or shore line of the swamp would be different from conditior existing in the central part of the swamp.

The Phalen seam was formed under uniform conditions in the central pa of the basin. Towards the edge of the basin, impurities are more prevaler and a thinning and splitting takes place in the seam. This seam is particularl well suited to microscopic study as it is the best known of the various seam in the field.

Formation of the Phalen Seam as Interpreted by the Microscopic Study

The microscopic components of coal are found in different proportior throughout the Phalen seam. The distribution of these components is (interest in that it throws light on the manner of origin and mode of formatio of this seam.

The microscopic study was confined solely to the Phalen coal in the centr part of the field, in Lingan, Bridgeport and Glace Bay basins. The equiv alents of the seam in the Morien basin to the west of Sydney Harbor wer not examined microscopically. The 13 pillars of coal which were studie show a gradual change in microscopic character from Sydney Harbor t Cape Percy. The coals in the pillars can be divided into three groups (Fig. 2

Group 1:— Coals characterized by high spore, high pyrite and low resi content. Pillars *A*, *B*, *C*, and *E* are of this type (Plate I, Figs. 2 and ; Plate II, Figs. 1–4).

Group 2:— Coal intermediate to Groups 1 and 3. This coal is a moderat spore, low-to-moderate pyrite and moderate-resin type, and is represente by Pillars *D*, *F* and *I* (Plate II, Figs. 5 and 6).

Group 3:— High-resin, high-pyrite, and low-spore coals. Pillars *G*, *H*, . *K*, *L* and *M* belong to this group (Plate III, Figs. 1–4).

Group 1 (Pillars A, B, C and E)

These pillars are located in the Lingan and western part of the Glace Bay regions. The spore content, the most distinctive feature of this group, is indicative of open-water deposition. Whether the spores were carried into the quiet pools of open water in the centre of the swamp by wind and water action is a question of some moment. Probably both forces would act if open-water conditions obtained for a considerable period of time, and, if there were no outside source of introduced plant matter, a cannel coal would be formed. A coal that appears to have a small spore content may have as many spores as a high-spore coal throughout the attritus, but, owing to the preponderance of woody tissue, may seem to be deficient in spore content. The proportion of spores increases in the middle of the pillars, which corresponds to the most stable conditions in the swamp. In this section of the Phalen seam, additional plant material was added to the swamp, and the result was a banded coal with a high spore content. The coal from this part of the field has had a large sale in the Boston and New York markets because of its high gas content and the illuminating qualities of the gas, properties which are a result of the spore content.

Associated with the spores are clear translucent bands of anthraxylon and humain. This would indicate that the process of decay had not been very active. In fact, open water in swamps is known to be a remarkable preservative of woody tissue. The greater thickness of the coal seam in the neighborhood of Pillars *C* and *E* could be accounted for in this manner. More plant matter would have been deposited in the part of the swamp least subject to open-water conditions. The greater degree of putrefaction, however, would tend to concentrate the vegetable matter, and would produce a coal which would show evidence of great decay. Therefore, a section of the swamp with a small supply of plant matter, and subject to little decay, could deposit as much embryonic coal as a section with a large supply of plant matter, but under conditions of great decay.

Resinous bodies, whose presence is a criterion of conditions of decay, are not common, but tend to increase in quantity towards the east. Opaque matter, which is possibly a product of extreme putrefaction, is not present in any quantity. The absence of pyrite and shaly matter is an indication of the inaccessibility of this region to the contaminating waters carried into the swamp. Fusain is scattered throughout the pillars in small lenticles which increase in quantity at the top and bottom of the pillars. The cell structure is well preserved in the fusain. The coal of this group is believed to have been deposited under the quiescent open-water conditions of the central part of the swamp. The components are best preserved here, producing a pure type of banded bituminous coal which is featured by its high spore content.

Group 2 (Pillars D, F and I)

The pillars were extracted from the central and western parts of Glace Bay basin. The coal of this group is intermediate in type to Groups 1 and 3.

The spore content decreases, while the resinous bodies and the disseminated pyrite increase, especially at the top and bottom of the pillars. The humain is not as translucent as in Group 1, and opaque matter is beginning to be more noticeable.

Coal of Group 2 is an intermediate stage in the passage from the more open-water conditions of the central part of the swamp, and the shore-line conditions that existed in the vicinity of Cape Percy.

Group 3 (Pillars G, H, J, K, L and M)

The pillars of this group were extracted from the eastern part of the Glace Bay region. The microstructure of the coal of this group is in marked contrast with that of Group 1; the abundance of resinous bodies is a distinctive feature. The accumulation of these resinous bodies would indicate that conditions of great decay existed in this section of the swamp. The woody tissue and other bodies less resistant to putrefaction were decomposed, and left a concentrated mass of humain and resin. The resins increase at the top and bottom of the pillars and in the vicinity of Cape Percy.

The high pyrite content of the coal from this section is an important economic factor, because it lowers the value of the coal. Pyrite is so abundant in places that it is disseminated in the form of lenticles. It shows the same distribution as the resinous bodies. Mineral and shaly matter also increase in the vicinity of Cape Percy. Shaly lenses are found in pillars *K*, *L* and *M*, and are more abundant at the top and bottom of the pillars. The pyrite and shaly matter were deposited at the time the seam was formed, and a progressive increase of those substances as Cape Percy is approached would indicate that their source had been in this region.

With the increase in pyrite and resins, the spores decrease. In most cases spores are present in small numbers, but as a rule they are practically absent. The amount of spores deposited in coal of Group 3 would be much less in proportion to the amount of woody matter deposited than in Group 1. Even the highly resistant spores show evidence of greater putrefaction in this part of the seam.

The anthraxylon is not of the clear translucent type found in Group 1. Pyrite bodies and opaque matter constitute impurities in it. Anthraxylon lenticles or bands are most abundant in this group, a result of the deposition of the more massive plant structures that grew near the edges of the swamp.

The humain of this group is quite different from the clear, structureless and translucent humain of Group 1. It is composed of numerous brecciated and cemented fragments of woody tissue possessing structural evidences of great decay. Opaque matter is intimately mixed in greatly increased proportions with the humain. With the increase of opaque matter, there is a similar increase in fusain. The fusain, however, is still more abundant at the top and bottom of the pillars. The well defined cell structure, characteristic of the fusain in the western part of the field, is replaced by a type in which the cell structure has been destroyed, with the formation of a black, structureless, opaque mass.

The degree of putrefaction shown by the various constituents of the coal would indicate that deposition had taken place near the edge of the swamp where conditions of decay were greatest. The presence of a stream that entered the swamp in the vicinity of Cape Percy would also be evidence of shore-line conditions. This stream would not only provide a source for the pyrite and other impurities in the coal, but would be an effective aerating agent, and would produce conditions favorable for extreme decay.

Certain constituents in the coal seam, among which are resins, opaque matter, fusain, shaly matter and pyrite, are more common at the top and bottom of the seam. The transgressive nature of the swamp was evidenced by the existence of a moving shore line, which resulted in a difference in the microscopic constituents of the coal at the top and bottom of the seam. During the period of greatest stability, when the Phalen swamp occupied its greatest extent, more spores were deposited in the central part of the basin. As a result, the coal in the middle section is richer in spores than that at the top and bottom of the seam.

Analysis of the Pillars

A series of complete proximate analyses of samples from the top to the bottom of each of the 13 pillars was made in the Fuel Research Laboratories of the Mines Branch at Ottawa. The average analysis of every pillar was plotted for the fixed carbon, volatile matter, ash and pyrite content. The divisions or groups of the pillars based on microscopic features were retained for a comparison of their chemical differences (Fig. 5).

The proportion of fixed carbon is quite uniform in Groups 1 and 2, not varying more than 4%, except in Pillar 1 where the coal is approaching Group 3 in type. Group 3 shows a progressive decrease in fixed carbon to the east.

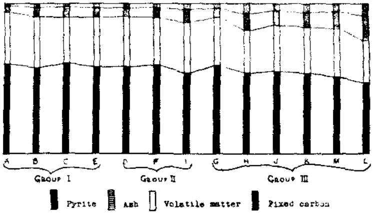


FIG. 5. *Variation in the average proximate analyses of coal pillars from the Phalen seam.*

The volatile matter does not show great variation because both resinous and spore coals are high-volatile coals. It is present in smaller quantities in the pillars that contain a high percentage of mineral matter and ash.

The ash content, as is to be expected, is highest in Group 3 and lowest in Group 1. The shaly matter and pyrite are responsible for the ash.

The sulphur content, as has already been observed, is highest in Group 3 and lowest in Group 1. Both the pyrite and ash content of the coal increase as Cape Percy is approached.

A chemical variation, as well as a change in the microstructure, is evident in the seam. The differences in the chemical nature of the coal are due mainly to the influx of impurities into the swamp in the vicinity of Cape Percy.

The pyrite content has been contoured (Fig. 2); its increase towards Cape Percy is a striking feature.

Gray (4, pp. 62–71) has propounded an interesting theory to explain the high sulphur and ash content of the coal in the vicinity of Cape Percy. He suggests that in this locality there was a spur of high ground projecting into the basin of deposition, thus causing a shallowing of the swamp and a deterioration of the coal in that part of the seam.

If this elevation existed during the period of coal formation, a definite thinning of the strata should be observed in the vicinity of Cape Percy. With this in mind, the stratigraphic intervals * between the Phalen and the Backpit, Emery, and Gardiner seams were calculated. These intervals were plotted throughout the field (Fig. 1). They show that there is no pronounced thinning of strata in the vicinity of Cape Percy. On the contrary, the stratigraphic intervals between the seams are greater in this part of the field than to the west. Therefore, no elevation could have existed in the vicinity of Cape Percy.

Future Mining Possibilities

It seems probable that the coal seams of the Sydney field extend so far out to sea that it will be impossible to mine them completely. As the Phalen seam dips gently seaward, and no serious folding movements have taken place, conditions appear very favorable for extensive mining development under the sea.

The mechanical difficulties in the mining of the undersea coal are not the only features to be considered. In operations of such magnitude, a deterioration in the quality of the coal would be disastrous. It is necessary to determine where the best and purest coal is located for future mining operations.

The purer coal of the central part of the basin is found to the north and west of the Bridgeport anticline, thus favoring future mining operations in this neighborhood. The high-sulphur and high-ash coals in the eastern part of the field should decrease progressively seaward as the source of the original impurities would have receded. Open-water conditions of deposition were probably more prevalent in this region; hence a purer type of coal was produced.

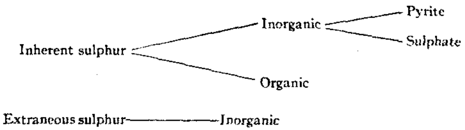
There is, therefore, every reason to believe that favorable conditions exist for an extensive undersea development of the Sydney coal field.

Sulphur in Coal

Sulphur is an important and universal constituent of coal, and in small proportions has little effect on it. Coals of a high sulphur content, however, have all their valuable properties modified, resulting in a coal of an inferior quality.

* *Estimated figures furnished by W. A. Bell, Geol. Surv. Can., 1932.*

Sulphur is found in several different forms in coal. It may originate either contemporaneously with, or later than, the coal. The former type may be termed inherent, while the latter may be called extraneous. The inherent sulphur can be of organic or inorganic origin, while the extraneous sulphur is always inorganic.



Inherent Sulphur

Inorganic Sulphur

Pyrite. The chief sulphur-bearing mineral in coal is pyrite. It is found in several different forms (1).

1. In bands parallel to the bedding.
2. In flat lenses.
3. In balls and rounded masses.
4. As disseminated bodies occurring in fine microscopic particles.

Sulphate sulphur. Sulphates are not abundant in coal; of these, gypsum is the commonest. Sulphates may be carried into a swamp and deposited there, or they may form as an oxidation product of pyrite.

Organic sulphur. This type of sulphur is mainly amicroscopic. According to Thiessen (9), bodies of disseminated pyrite are sometimes of organic origin.

Extraneous Sulphur

This type of sulphur, chiefly in the form of pyrite, is introduced after the coal has been formed. It crosses the bedding and penetrates the joints and fractures in the coal, forming plates and sheets of pyrite. The roof of the coal seam containing extraneous pyrite should be closely examined in order to determine the extent of fracturing. Highly fractured roofs would permit the penetration from above of sulphide-bearing solutions and deposition of sulphides in the seam. An undisturbed roof would indicate a coal relatively free from this type of pyrite.

Origin of Sulphur in Coal

The sulphur in coal may be formed by the infiltration of marine waters or sulphur solutions into the swamp. Bacterial and plant sulphur are additional sources.

Marine Conditions

The high percentage of sulphur in certain coal basins is attributed to marine submergence of the coal-forming peat deposits. A relation between

high-sulphur coals and roofs of marine origin containing remains of marine invertebrates has been observed (2). Whether the iron and sulphur salts in sea water are present in proportions sufficient to produce pyrite when precipitated by the decaying organic matter is open to question. It is noteworthy that most of the high-sulphur peats are salt-marsh peats.

Sulphur Solutions

Solutions bearing sulphur and iron may be carried into a swamp and the sulphur and iron precipitated. The iron may come from the iron-containing minerals of the surrounding land, while gypsum beds may provide an adequate source for the sulphur.

According to Harder (5), during the process of putrefaction of the vegetable debris, carbon dioxide and marsh gas are evolved. The marsh gas attacks any calcium sulphate with the evolution of hydrogen sulphide. Marsh gas also reduces the iron salts of the vegetable debris producing ferrous sulphide. Coal-forming material containing a local excess of ferrous sulphide would produce pyrite bands (5, pp. 74-75, 82-84).

Iron and sulphur solutions are precipitated by organic products of decay. In fact, it is possible that in the swamp the iron may be in the form of soluble salts of certain humic acids. Iron carbonate and limonite are often found in peat bogs which may form pyrite by the decomposition of calcium or magnesium sulphate with the liberation and removal of calcium carbonate. The iron carried into the swamp may be precipitated directly as a black colloidal hydrate which, in the presence of hydrogen sulphide under reducing conditions, is changed to hydrated ferrous monosulphide, and this in turn is changed to pyrite (5, pp. 74–75, 82–84). The rate at which these solutions enter and are precipitated in the swamp varies greatly, producing layers of high and of low sulphur content.

Bacterial Sulphur

Sulphur and iron bacteria are well known in nature. These organisms obtain vital energy from the sulphur and iron compounds.

Certain sulphur bacteria produce hydrogen sulphide (5, pp. 74–75, 82–84) by the decomposition of the sulphur-bearing proteins. Other sulphur bacteria have the ability to reduce sulphates. In the presence of decaying organic matter they take oxygen from the sulphates and form sulphides. Another type acts directly on free sulphur to form hydrogen sulphide which acts upon ferrous salts in solution.

Iron bacteria are also active in breaking down iron salts in solution. They store ferric oxide in their cell walls and under favorable conditions produce deposits of bog-iron ore. An iron oxide ball built up by these bacteria, if lying in coal, may under suitable conditions be changed into pyrite.

Thiessen (9, pp. 919–923) has recorded the presence of pyrite-producing bacteria in peat. Some of the microscopic disseminated pyrite found in higher-rank coals is possibly of this origin.

Plant Sulphur

Sulphur in plants consists of two types, protein sulphur and non-protein sulphur. The sulphur in the proteins is set free during putrefaction. There is more than enough sulphur in plant material (9, pp. 919–923) to supply all the sulphur in coal, considering that the sulphur content remains constant and the plant matter decreases in volume in the process of coalification.

Plant sulphur in coal is amicroscopic and can be determined only by chemical tests. Two types of plant sulphur are distinguished in coal, humic sulphur and resinic sulphur.

Sulphur in the Phalen Seam

Types and Distribution

The sulphur content is an important feature of the Phalen seam. The sulphur percentage is so high in certain sections that it has a deleterious effect on the coal. The sulphur is found in organic and inorganic forms. The organic sulphur is of course amicroscopic, and is determined only chemically. The inorganic sulphur is mainly pyrite.

The pyrite ranges from one micron to macroscopic dimensions. It is distinctly of the disseminated type, and in places it becomes so abundant that lenticles of disseminated pyrite are formed. The pyrite is in the form of small, oval, opaque bodies which show the characteristic brassy yellow of pyrite in reflected light. The pyrite is most abundant in the attritus although pyrite bodies are also found in the anthraxylon (Plate III, Fig. 4). No pyrite was detected in the fusain.

The chemical analyses and the microscopic examination of the Phalen seam reveal a variation in the pyrite content of the seam both horizontally and vertically. Pyrite increases in the top and bottom of the seam, and this relation holds throughout its whole extent. The gradation coincides directly with the variation in microstructure of the seam. A high-spore coal is essentially a coal low in pyrite (Group 1). An increase in pyrite is noticed in the section of the seam that has a moderate spore and resin content (Group 2). The resin coals are the ones which are richest in the percentage of pyrite (Group 3 (Fig. 2)). In Group 3, pyrite is very evident, producing a high percentage of sulphur in the coal. The pyrite content of the coal progressively increases as Cape Percy is approached.

Analyses were made in the Fuel Research Laboratories at Ottawa for the types of sulphur found in the Phalen seam. Pyritic sulphur, organic sulphur and sulphate sulphur were determined. Sulphur in the low-sulphur coal is mainly organic, while in the high-sulphur coal it is found in the form of pyrite. The sulphate content, for the most part gypsum, is negligible.

Origin

The Phalen seam was formed under strictly terrestrial conditions. Therefore infiltration of marine waters would not be a factor in the formation of

the pyrite in the seam. Bacterial and plant sulphur, although contributing to the total sulphur, could not account for the high pyrite content and its peculiar distribution in the seam.

That sulphate and iron solutions entered the swamp and formed pyrite, offers the most satisfactory explanation for the origin of the pyrite. Iron, in various forms, and gypsum deposits are found in various localities throughout the Sydney coal field.

The pyrite distribution would indicate that the source of the pyrite was a river carrying sulphate- and iron-bearing waters which entered the swamp in the vicinity of Cape Percy. The stream came into the swamp from the westward rather than the eastward side of Cape Percy. The pyrite content in the Phalen seam decreases to the west. In the Morien basin the sulphur is highest in that part of the Gowrie seam nearest to Cape Percy. Evidence obtained with the microscope tends to confirm the conclusion that there was an influx of sulphur- and iron-bearing waters in the vicinity of Cape Percy, that is to say, the higher sulphur content of the Phalen seam in this neighborhood is a strictly local condition associated with approach to the edge of the original coal swamp.

There was apparently a greater influx of the sulphur- and iron-bearing waters when the swamp was first formed, which decreased when conditions of swamp formation were more uniform and the swamp had reached its maximum extent. In the closing stages of swamp formation, when one set of conditions was changing to another, there was another increase in the influx of these waters. A coal high in pyrite at the top and bottom of the seam was thus produced.

The coal nearest the source of the sulphur and iron is naturally highest in pyrite content. The percentage of pyrite is highest where decay had been most active in the swamp. The organic products of decay were effective precipitating agents of the iron- and sulphur-bearing waters.

There is, therefore, no reason to expect an increase in the proportion of pyrite as the seam is worked progressively seaward. On the contrary, the pyrite content should decrease. The best type of coal for future development would be found to the west of the coal comprised in Group 3.

Spore Correlation in the Sydney Coal Field

Correlation of the strata and coal seams in the Sydney field is fraught with difficulties. No reliable correlation of the coal seams can be based on a comparison of thickness and lithology of the individual beds, owing to the nature of flood-plain deposition. The subdivision of the field by structural and erosional features, as well as by independent mining operations, has given rise to different names for the same seam throughout the field. A knowledge of the extent of the various seams in the field is of great importance to the mining companies as a guide for future mining operations.

The recently developed method of correlating coal seams by means of the spore types contained in the coal and by their distribution in the seams is of

interest. The method has already proved of value in coal areas where correlation by stratigraphic and ordinary paleontologic methods is difficult. As a preliminary step, the spores in the Phalen seam were examined and classified into various groups and types. As yet, a microscopic examination of the spores in the other seams of the field has not been initiated. The writer hopes that the following account will furnish a basis for a future investigation which has great possibilities.

In order that a spore may be of value in correlation, it must have a definite physical appearance by means of which it may be easily recognized. In other words, this type of correlation is based on the theory that there was a change in the type of plant in the intervals between the depositions of the various coal beds. The change would manifest itself in differences in types of spores.

In England it was found (7, p. 8) that no particular type of spore would serve to identify a seam by its presence. The varying concentration of several types of spores in different horizons in the seam, however, proved characteristic. The statistical method of fossil distribution, common in palaeontology, was used as the main basis for the correlation of English coal seams.

The method of spore counts may be satisfactory in seams where uniform conditions prevailed over great areas, but where conditions changed, this method is not adequate. In the Sydney field, conditions were not uniform,

TABLE II

MEGASPORES IN THE PHALEN SEAM (FIG. 3)

Spore	Figure	Size, μ	Description	Distribution
1	A	110–350 Average 170	Smooth-walled	Most abundant megaspore. Chiefly in Group 1
2	B	250–300	Smooth-walled with pointed ends	Abundant in Groups 1 and 2
3	C	200–500	Has a striated tuft-like appendage	Present only in Groups 1 and 2
4	D	100–500	Very slender, smooth-walled	Practically confined to Group 1
5	E	220–270	Smooth, double-walled	Confined to Group 1
6	F	240–340	Thick, with tubercles	Not common, and confined to Group 1
7	G	200	Serrated	Rare
8	H	220	Squat, bulky	Unique
9	I	150	Bulky, with square tubercles	Occurs in Pillar K

and it has already been pointed out that the spore content of the Phalen seam varies greatly. In the Lingan basin, the coal contains enormous numbers of spores, chiefly microspores. To the east, a progressive decrease in the quantity of spores has been noted, and in places they may be practically absent. Graphical methods of comparing the quantity of spores in different parts of the field would thus be valueless. The only remaining satisfactory method would be the detection of singularly characteristic spores in the coal bed. Spores of this type have been found in the United States.

The spores in the Phalen seam are as a rule smaller than the types of spores found in the English and American coals. The microspores range in length from 6 to 40 μ, and megaspores from 100 to 500 μ.

TABLE III

MICROSPORES IN THE PHALEN SEAM (FIG. 4)

Spore	Figure	Size, μ	Description	Distribution
1	A	Average, 14	Smooth-walled	Commonest microspore. Found often in layers. Most abundant in Group 1
2	B	23–40	Nodular	Abundant in Groups 1 and 2 and uncommon in Group 3
3	C	Average, 7	Small, thick, with smooth and nodular varieties	Present mainly in Group 1
4	D	Average, 13	Serrated	Present only in Groups 1 and 2
5	E	Average, 18	Pointed ends with smooth, nodular and serrated varieties	Most abundant in Group 1 and least in Group 3
6	F	Average, 6	Very slender, small	Predominant in Group 2
7	G	Average, 13	Salver-shaped	Most abundant in Group 1, decreasing progressively eastward
8	H	Average, 26	Thin-walled, spinose	Uncommon and found chiefly in Group 3
9	I	Average, 28	Stout, with fleshy tubercles	Found mainly in Group 3. Uncommon
10	J	Average, 13	Notched	Uncommon and found only in Group 3
11	K	Average, 11	Smooth, double-walled	Sparingly in Group 1

Acknowledgments

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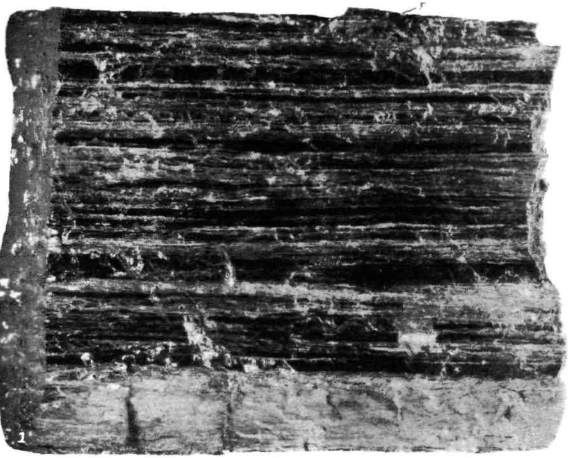
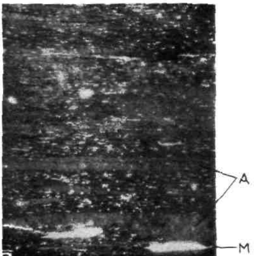




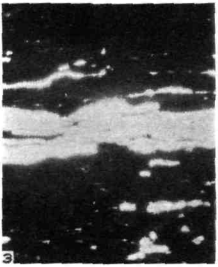
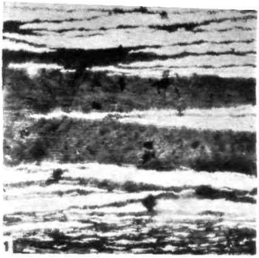
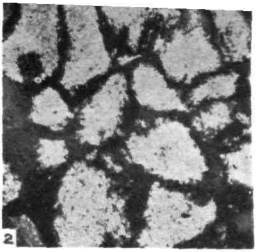
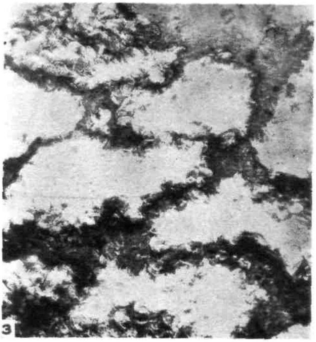
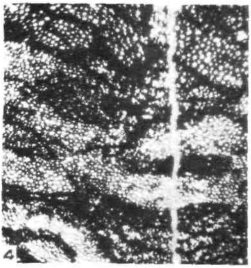


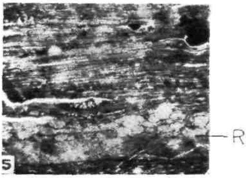
FIG. 1. *Coal from Pillar M, Phalen seam. The banded character of the anthraxylon, or bright coal, and the attritus, or dull coal, is clearly shown. A lenticle of fusain (F) is found at the top. A layer of carbonaceous shale can be seen at the bottom of the specimen.* $\times \frac{1}{2}$. FIG. 2. *Pillar B, Section 10. Microspores are abundant. The megaspores (M) at the bottom are of the sharp-pointed variety described as Type 2. A few thin bands of anthraxylon (A) are clearly distinguished.* $\times$ *31*. FIG. 3. *Pillar E, Section 22. The layer of megaspores is composed of the smooth-walled Type 1.* $\times$ *61*.











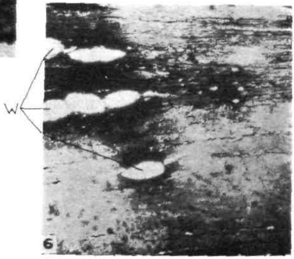
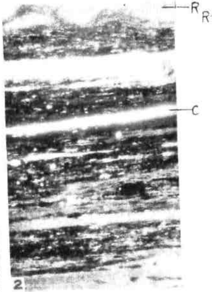
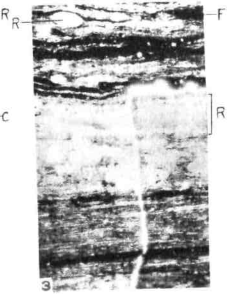


FIG. 1. *Pillar E, Section 18. Microspores belonging to Type 1 compose the layers. The layers may be remains of sporangia flattened by pressure. A forking anthraxylon lenticle separates some of the layers.* × 33. FIG. 2. *Pillar E, Section 18. Possibly a horizontal section of microsporangia as shown in Fig. 1. The circular outline of the individual microspores can be distinguished in places.* × 29. FIG. 3. *Pillar E, Section 18. Same section as Fig. 2 only more highly magnified. In places the tetrad markings of the microspores can be distinguished. The arrangement of the spores suggests that these are the remains of sporangia.* × 117. FIG. 4. *Pillar E, Section 4. Fusain showing well-preserved cell structure. The cellular spaces are empty.* × 33. FIG. 5. *Pillar D, Section 5. The attritus is composed of relatively translucent groundmass with some opaque matter. A layer of resins (R) indicates a stage of extreme putrefaction. A few microspores and cuticles are scattered throughout the attritus.* × 33. FIG. 6. *Pillar I, Section 11. Microspores are practically absent. Resinous bodies are abundant but are not clearly shown. Waxes (W) of a lemon-yellow color are distinctive. Opaque matter is fairly common.* × 73.





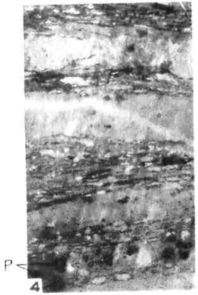


FIG. 1. *Pillar G, Section 5. Microspores are practically absent. The groundmass is composed of minutely comminuted fragments of woody tissue or humain with some opaque matter. Structureless fusain is found in lenticles. Resinous bodies are found throughout the section.* × 66. FIG. 2. *Pillar G, Section 11. Opaque matter and humain make up most of the attritus. A cuticle (C) with a serrated border is shown. At the top of the section several large globules of resins (R) are seen.* × 33. FIG. 3. *Pillar M, Section 18. The coal is of an impure nature. Opaque matter and fusain are present in large proportions. The attritus apparently was formed under conditions of great decay. Resins (R) are abundant.* × 33. FIG. 4. *Pillar K, Section 11. Disseminated pyrite (P) is shown to be mainly confined to the attritus.*

investigation have proved of great assistance. He is indebted to Dr. W. A. Bell of the Geological Survey of Canada, who furnished him with information and notes, as yet unpublished, on the general geology of the field. Further, an expression of sincere thanks is due to Mr. R. E. Gilmore and Mr. J. H. H. Nicolls of the Fuel Research Laboratories, Ottawa, for helpful co-operation and for the analyses presented in this work. Finally, the constructive criticism of Mr. F. E. Lathe, Director, Division of Research Information, National Research Council of Canada, was of great value in the preparation of the manuscript for publication.

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THE RESIN OF WHEAT STRAW[1]

By Léo Marion[2]

Abstract

A study of the resin extracted from wheat straw by methanol-benzene (50–50 by volume) has shown that about one-quarter of it (or 1.5% of the straw) consists of a mixture comprising an oil, free fatty acids, a wax and a small quantity of an essential oil. On saponification, the oil yields sitosterol and a mixture of fatty acids (palmitic, stearic, oleic, linolic and lignoceric acids were identified), while the wax yields ceryl alcohol, sitosterol, palmitic and cerotic acids.

The remainder of the resin (4.7% of the straw) consists mostly of lignin, but contains in addition small quantities of phytosterolin, sterol ester, *i*-inositol and potassium nitrate.

A previous paper by the writer (10) dealt with the isolation of lignin from the straws of wheat and other cereals. In the isolation of lignin from wheat straw, the latter was first extracted with a mixture of methanol and benzene, the extract yielding a sticky, semifluid mass generally designated as resin. It represented 6.22%* of the weight of dry straw. Comparatively little is known concerning this resin beyond the fact that it comprises a material of waxy consistency (5).

As early as 1869 Radziszewski (14) isolated substances of waxy consistency from cereal straws but did not investigate them. Later König (8, 9), working with rye straw and oat straw, stated that he had effected a separation of the wax from the fat which he considered as consisting of tristearin, tripalmitin, and triolein together with a hydrocarbon, ceroten.

The petroleum ether extract of wheat straw contains, besides glycerides, an appreciable proportion of wax. Concerning this wax, Heuser (5), in a cursory study, mentioned that it is light yellow and that it forms 1.18% of the weight of dry straw. He had not, however, effected any separation of the fat and wax, and was dealing with a mixture.

It is known that the oils extracted from the various parts of the wheat kernel such as the germ, inner endosperm and the bran, differ markedly from one another (2), and the dissimilarity has been found to extend to the oil present in the stalk or straw which is identical with none of the former.

About one-quarter of the resin, or 1.5% of the weight of straw, was found by the author to be extractable with petroleum ether and consists of a mixture of a fat or oil, and a wax, together with a small quantity of essential oil. The oil, when separated from the wax, has the constants recorded in Table I. For purposes of comparison the constants of wheat-germ oil, determined by Jamieson and Baughman (7), are also given in the same table.

[1] *Manuscript received December 3, 1934.*
Contribution from the Division of Chemistry, National Research Laboratories, Ottawa, Canada.

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**This was reported previously (10) as 11.4%. It has now been found that extraction with methanol-benzene for 24 hr. removed 6.22%, while a second and a third extraction, each of 24 hr., with methanol removed a further 3.5% and 0.8%, totalling 10.5%. The methanol extracts were found to be largely soluble in water. They were not further investigated.*

Free acids are present in the straw oil in rather large quantities and consist of palmitic, stearic and lignoceric acids. No resin acids whatsoever are present. The total acids obtained after saponification amount to 42.0% of the fat. They include oleic, linolic, stearic, palmitic and lignoceric acids together with a small quantity of a waxy substance which is insoluble in alcohol. In the unsaponifiable, which is present in considerable quantity, no alcohol other than sitosterol, m.p. 137° C,* could be detected. Since Anderson, Shriner and Burr (1) have shown the wheat-germ sterol to be a mixture, the present sterol is also probably heterogeneous: it was not further examined, although it was purified through its acetate.

TABLE I

PHYSICAL AND CHEMICAL CONSTANTS OF WHEAT-STRAW AND WHEAT-GERM OILS

	Wheat-straw oil	Wheat-germ oil
Refractive index	(26° C.) 1.4952	(20° C.) 1.4762
Acid value	35.6	7.6
Saponification value	86.6	186.5
Iodine value	(Waller) 57.5	(Hanus) 125.6
Reichert-Meissl value	1.07	0.2
Polenske number	1.35	0.35
Unsaponifiable, %	49.6	4.7
Saturated acids, %	12.1	13.3
Unsaturated acids, %	29.9	75.3
Insoluble in alc., %	3.2	—

The alcohol-insoluble fraction after purification and distillation consisted of small shining flakes, m.p. 70° C. On hydrolysis it produced cerotic acid, and probably consists of impure ceryl cerotate. Both the analytical figures and the production of cerotic acid on hydrolysis point to that conclusion, although its insolubility in alcohol does not agree with the recorded properties of that ester.

The wax, which is a soft, white solid (saponification no. 64.3; iodine value, 25.8), yielded ceryl alcohol (acetate m.p. 64° C.), a mixture of palmitic and cerotic acids and sitosterol, m.p. 137° C. Cerotic acid has recently been reported in rye straw (13) without, however, any study of the wax having been made. The phosphorus content of the wax amounts to 0.042% P_2O_5. Although this cannot be taken as an estimation of the phosphatides present, it may possibly be assumed to indicate the presence of a trace of the latter in wheat straw.

In addition to the foregoing substances the extract seems to contain a sterol ester. It was not possible to isolate the ester in a pure condition but, after saponification, a crystalline sterol which yielded an acetate, m.p. 131° C. was isolated. The presence of a sitosterol ester in wheat-germ oil has been reported by Ball (2), although the evidence is rather circumstantial. Ball, who also experienced a great deal of difficulty in purifying his product, failed to isolate the sterol in crystalline form and detected it by colorimetric tests only. In the present instance, the isolation and characterization of the sterol

*All melting points are corrected.

seem to indicate the presence of a sterol ester in wheat straw and might perhaps support Ball's evidence for the presence of such a compound in wheat-germ oil.

A phytosterolin, characterized through its acetate, m.p. 159° C., and by Whitby's color test (15), has also been isolated from the extract. It has been further characterized by admixture with the sterolin from *Adlumia fungosa* (11), which failed to depress its melting point, and by hydrolysis. A sterolin has recently been isolated by Nakamura and Ichiba (12) from the wheat embryo, and the properties recorded by them are in agreement with the foregoing. The two substances have identical melting points and the sterols obtained on hydrolysis in each case melt at 137° C. Nakamura and Ichiba prepared an acetate which melts at 167° C., whereas that obtained in this case melts at 159° C., but this may be owing to the presence of a trace of impurity, the quantity on hand being too small to permit further purification. A mixture of this acetate with that obtained from the sterolin of *A. fungosa* melts at 157° C.

The resin contains a large proportion of lignin which is readily obtained after separation of the fat and wax. Some of the lignin isolated is no longer soluble in methanol or benzene. Since an appreciable amount of lignin is generally dissolved from straw by an organic solvent only in the presence of a catalyst, the removal of these quantities of lignin by methanol-benzene would tend to show the presence in straw of a small proportion of lignin which is not as firmly held as the major part of it. It has been shown that alcohol-benzene will remove small quantities of lignin even from ground wood (6), although lignin is normally removed from straw by much milder methods than those required to remove it from wood. Analogous cases of readily extractable lignin are repeatedly found in the literature.

Potassium nitrate (3%) and a small amount of *i*-inositol, m.p. 224° C., have also been found to occur in the wheat-straw extract.

Experimental

Wheat straw, ground in a Wiley mill, was thoroughly extracted with a mixture of methanol and benzene (50% by volume). This treatment removes 6.22% of the weight of the straw and yields an extract which, after removal of the solvent, consists of a dark brown, soft, sticky resin having an iodine absorption value of 28.6. Approximately 0.5 kg. of resin was used for the present work. With the aid of methanol the resin was mixed with shredded asbestos and the methanol removed on the steam bath. The hard cake produced was crushed and extracted in Soxhlets successively with petroleum ether, ether, chloroform, ethyl acetate, and methanol, the residue being then extracted in a flask with water and dilute sodium hydroxide.

Petroleum Ether Extract

After removal of the solvent this extract amounted to 133 gm. and consisted of a soft, resinous solid. It was suspended in water and distilled for five hours in a strong current of steam. The aqueous distillate, through

which were scattered small globules of oil, was shaken with several portions of ether. The extract, after drying over anhydrous sodium sulphate and removal of the solvent, yielded a small quantity of a light-brown oil which, when distilled under diminished pressure (b.p. 75–120°C./4 mm.), was obtained as a clear yellowish oil having a refractive index (22.5° C.) of 1.4862.

Isolation of Free Fatty Acids

The extract which had been steam-distilled consisted of a mixture of fat, wax and free acids. It was collected in ether and shaken with several portions of 5% sodium bicarbonate solution. The combined sodium bicarbonate solutions were washed with ether and acidified, the separated acids being collected in ether. The solution was dried over calcium chloride and the ether was evaporated. A thick oil was left in appreciable quantity. The relatively large proportion of free acids was indicated by the high acid number of the fat (35.6). The oil was esterified with methanolic sulphuric acid by the preferential method of Wolff and Scholze (16) which is known to esterify the fatty acids while it leaves the resin acids in the free state. No acidic residue having been left in this case it can be concluded that resin acids are absent and that the free acids belong entirely to the fatty series.

The methyl esters of the acids occurring in the free state, when subjected to distillation under diminished pressure, yielded three fractions: Fraction 1 consisted of an oil (b.p. 140–147° C./3.5 mm.), which crystallized on cooling and melted at 23–24° C.; when saponified with alcoholic potash this ester yielded an acid which was recrystallized from a mixture of alcohol and ethyl acetate, m.p. 62° C., and was found to be identical with palmitic acid. Fraction 2 consisted of a slightly yellow oil (b.p. 165–169° C./3 mm.) which, on saponification, yielded stearic acid crystallizing from alcohol at 0° C. in small white masses. Fraction 3 was crystalline and, on saponification, produced lignoceric acid which was recrystallized several times from alcohol, m.p. 73–74° C. Equivalent: calcd. for $C_{24}H_{48}O_2$, 368; found, 375.

In order to determine the constants of the oil and determine the unsaturated acids a fresh lot was prepared by extracting wheat straw with light petroleum ether. The solution was concentrated, treated with charcoal and filtered. The rest of the solvent was removed in an atmosphere of carbon dioxide and the residue dissolved in sufficient ether to make a 25% solution. A small quantity of a white insoluble substance was filtered and washed with ether; it amounted to 7.4% of the petroleum ether extract. To the combined filtrate and washings was added twice its volume of warm acetone which caused the precipitation of the wax. The well stirred mixture was kept in the ice chest overnight, then filtered and washed with a small volume of ether-acetone (1–2 vol.). The filtrate, after the solvent was distilled off in an atmosphere of carbon dioxide, yielded 45.4% of a yellow oil. The white granular solid on the filter contained the wax and any phosphatides; it amounted to 47.2%.

Saponification of the Glyceride

After the determination of the various constants which are given in Table I, the bulk of the oil was saponified with alcoholic potash and separated into unsaponifiable and acidic portions.

Unsaponifiable Fraction

Quantitatively, the unsaponifiable matter was determined on a sample of the oil by the Kerr-Sorber method as modified by Hertwig, *et al.* (4). The main bulk of the unsaponifiable matter combined with that obtained in the quantitative determination was boiled with alcohol, and the crystalline product recrystallized from that solvent several times. No fatty alcoholic constituent was present, and the sterol, purified by the preparation of its acetate followed by hydrolysis, was obtained as glistening flakes, m.p. 137° C.

Isolation of the Fatty Acids

The acids, separated from the unsaponifiable matter, amounted to 42.0% of the oil. They were dissolved in alcohol, filtered from a small amount of insoluble (3.2%) which will be referred to further on, and separated into saturated (12.1%) and unsaturated acids (29.9%) by the lead-soap-alcohol method (3, p. 376). The percentages given are all based on the amount of fat. From the saturated acids palmitic, m.p. 62° C., and stearic acids, m.p. 68° C., were obtained by fractional crystallization, the former being present in appreciably larger quantity than the latter. On oxidation with cold, dilute permanganate by Lapworth and Mottram's method the unsaturated fraction yielded sativic acid, m.p. 173.5° C., and dihydroxystearic acid, m.p. 127° C., proving the presence of linolic and oleic acids.

A sample of wax (0.6445 gm.) was digested with concentrated nitric acid and its phosphorus content determined by converting into ammonium phosphomolybdate, dissolving in standard alkali and titrating the excess. The wax contains 0.042% P_2O_5.

The very thick, oily substance which had been separated from the fatty acids through its insolubility in alcohol was found to be soluble in ether, dioxane and benzene, from which solvents it separated on addition of alcohol, in a semicrystalline state. After repeated separations from dioxane-alcohol, the substance was mixed with methanolic sulphuric acid, enough benzene added to bring about complete solution, and the solution boiled on the steam bath for three hours. This treatment seemed to effect the removal of some impurity and the substance was then fractionated under reduced pressure. Fraction 1, which was a colorless oil, b.p. 240° C./3.5 mm., crystallized from alcohol in very small plates, m.p. 60–61° C., which could not be further identified owing to paucity of material. Fraction 2, a white crystalline substance, was recrystallized from ethyl acetate from which, by the cautious addition of alcohol, it separated as white flakes, m.p. 70° C. Calcd. for $C_{52}H_{104}O_2$: C, 82.02; H, 13.78%. Found: C, 83.33, 83.68; H, 14.28, 14.28%. When saponified with alcoholic potash in the presence of some

benzene, this substance yielded a crystalline acid, m.p. 79–80° C. Calcd. for $C_{26}H_{52}O_2$: C, 78.70; H, 13.22%. M.W. 396. Found: C, 78.21, 78.41; H, 13.07, 13.00%. M.W. (Rast) 348, 348. The analytical figures obtained for the substance and the acid derived from it are, therefore, in fair agreement with those calculated for ceryl cerotate and cerotic acid.

Saponification of the Wax

The wax, as already mentioned, amounted to 47.2% of the petroleum ether extract, and consisted of a soft white solid. It was saponified by refluxing with sodium alcoholate for one hour. To the solution, cooled to about 70° C., sufficient water at 50–60° C. was added to make the concentration of alcohol approximately 85% and the resulting solution was extracted several times with ligroin (60–90° C.). After washing the ligroin extract with four portions of 85% alcohol, and distilling the solvent under slightly diminished pressure, the residue (48% of the wax) was dissolved in boiling acetone and the solution was filtered while hot and allowed to cool. It deposited a white crystalline substance, m.p. 76° C., which, distilled under diminished pressure (b.p. 198–200° C./3 mm.) and recrystallized from ethyl acetate, formed pearly scales, m.p. 75° C. Calcd. for $C_{26}H_{54}O$: C, 81.67; H, 14.25%. M.W. 382. Found: C, 83.55, 83.67; H, 14.44, 14.43%. M.W. (Rast) 351, 362. This substance therefore consists of ceryl alcohol. Boiled with acetic anhydride in the presence of a trace of pyridine, it forms the characteristic crystalline acetate, m.p. 65° C.

Isolation of Sitosterol

The acetone filtrate from ceryl alcohol was evaporated and the residue dissolved in boiling alcohol. On cooling, the solution deposited a crystalline sterol, m.p. 137° C., which was not further studied.

Isolation of Palmitic and Cerotic Acids

To the alcoholic solution which had been extracted with ligroin, a slight excess of a solution of hydrogen chloride in 85% alcohol was added and the liberated acids extracted by shaking again with several portions of ligroin. The residual alcoholic solution was then diluted with much water and extracted with ether. This extract yielded a light-brown resinous deposit which became crystalline on standing: it amounted to 12% of the wax but could not be further identified. After removal of the solvent from the ligroin solution, the residue (40% of the weight of wax) was boiled with methanolic sulphuric acid and the esters were fractionated under diminished pressure. Two fractions were collected, one boiling at 125° C./3.5 mm., melting at 24–25° C., and identified as methyl palmitate, the other yielding a solid which, after crystallization from alcohol, melted at 68° C. On saponification, this ester produced an acid, m.p. 83–84° C., which, by a mixed melting point determination and analysis, was proved to be identical with cerotic acid. Calcd. for $C_{26}H_{52}O_2$: C, 78.70; H, 13.22%. Found: C, 80.97, 81.06; H, 13.40, 13.44%.

Ether Extract

This extract, amounting to 55.5 gm., was digested with cold ether and the insoluble filtered and washed with ether. The combined filtrate and washings were evaporated to a convenient bulk and extracted successively with 5% aqueous solutions of sodium bicarbonate and of sodium hydroxide. From the residual ether solution nothing definite could be obtained.

The sodium bicarbonate solution on acidification yielded an oil which was converted to the methyl ester; the latter was fractionated under reduced pressure. It yielded a light-yellow oil, b.p. 140–167° C./4 mm. The former, when saponified produced a very small quantity of a crystalline acid, too small to permit further identification.

The sodium hydroxide extract yielded only a small quantity of uncrystallizable gum.

Isolation of Phytosterolin

That portion of the ether extract which was sparingly soluble in cold ether was dissolved in a little dioxane (charcoal) and an equal volume of boiling alcohol added to the concentrated solution. On cooling, a precipitate formed which obviously consisted of a mixture. Several repetitions of this process finally effected the separation of the mixture into a soft, waxy substance and a crystalline compound which could be recrystallized from dioxane-alcohol, m.p. 291–292° C. Calcd. for $C_{33}H_{56}O_6$: C, 72.25; H, 10.21%. Found: C, 72.43; H, 10.25%. This substance therefore is a phytosterol glucoside, and admixture with the similar compound obtained from *A. fungosa* (11) failed to alter the melting point. In Whitby's color reaction it gave a positive test. When boiled with acetic anhydride and a trace of pyridine it forms a crystalline acetate which, recrystallized from methanol, melts at 159° C., and when admixed with the acetate produced from the sterolin of *A. fungosa* (m.p. 157°), melts at 157° C. Furthermore, when hydrolyzed in amyl alcoholic hydrogen chloride, according to the usual method, the sterolin yields a sterol, m.p. 137° C., and glucose, identified as its osazone.

Isolation of a Sterol Ester

The soft, amorphous substance separated from the sterolin was, after repeated recrystallizations from alcohol, still colored brown. It was then refluxed with methanolic sulphuric acid. This treatment removed a small amount of impurity, but did not bring about crystallization of the substance which was eventually boiled with alcoholic potassium hydroxide. The solution was filtered while hot from a little insoluble material and an equal volume of water added. This caused the formation of a slight precipitate which became flocculent as the flask cooled to room temperature. Shaking with several portions of ether removed the precipitate, and the clear solution was acidified and again extracted with ether. The latter extract on evaporation left a small quantity of oil which was esterified, but the yield of product was too small to permit distillation. The ether extract of the neutral substance

after evaporation of the solvent left a crystalline residue which, recrystallized from methanol, melted at 134–135° C. This was boiled with acetic anhydride containing pyridine and the acetate, crystallized from methanol, melted at 130–131° C. Calcd. for $C_{27}H_{46}O.COCH_3$: C, 81.31; H, 11.22%. Found: C, 81.02; H, 11.22%. It gave positive tests in Whitby's color reactions *B* and *C* (15).

Chloroform Extract

After distillation of the solvent, the extract left a resinous mass (36.9 gm.) which was separated by shaking with chloroform into a sparingly soluble part *A* and a part *B*, readily soluble. The chloroform solution of the latter was extracted first with a 5% aqueous solution of sodium bicarbonate and then with a 5% solution of sodium hydroxide. In the course of the extraction with sodium bicarbonate, an insoluble gum *C* separated. This was collected. Nothing but a trace of untractable material could be obtained from the sodium bicarbonate solution.

The sodium hydroxide solution on acidification yielded a bulky precipitate of lignin, which after purification weighed 22.7 gm.

The gum *C* which had separated from the chloroform solution was dissolved in dilute caustic and the filtered solution acidified. Lignin was thus precipitated as a light-brown, granular solid. After repeatedly washing by decantation it was filtered and dried (3 gm.).

The part *A* of the extract which was sparingly soluble in chloroform was boiled with methanol and the mixture filtered while hot. After repeating this twice with fresh portions of methanol, the filtrates were combined and evaporated. The residue was dissolved in dilute caustic and the filtered solution, on acidification, yielded a small quantity (0.9 gm.) of lignin.

From the methanol-insoluble part of Fraction *A*, 8.1 gm. of lignin was isolated in a similar way. Hence 94% of the chloroform extract consisted of lignin.

Ethyl Acetate Extract

According to the difficulty or ease of solubility of the various constituents in ethyl acetate, this extract was separated into two fractions. From an ethyl acetate solution of the readily soluble part of the extract four fractions were obtained, *i.e.*, *A*, soluble in 5% sodium bicarbonate; *B*, soluble in 5% sodium hydroxide; *C*, the neutral residue; and *D*, a gum which separated in the course of the extraction with dilute sodium hydroxide. From Fraction *A*, an oil was precipitated on acidification. It was dissolved in ethyl acetate, dried and esterified with methanolic sulphuric acid. When fractionated under diminished pressure, the ester yielded a light-brown oil, b.p. 155–180° C./4 mm., and a trace of a thick brown oil, b.p. 180–240° C./4 mm. The former was saponified but the product could not be crystallized and was discarded. Nothing tractable could be obtained from Fractions *C* and *D*, but Fraction *B* yielded lignin and so did that part of the original extract which was sparingly soluble in ethyl acetate (4.7 gm.).

Methanol Extract

The solvent was largely distilled and the clear, concentrated extract allowed to stand several weeks at room temperature during which it gradually deposited a crystalline mass which was filtered on a Buchner and washed with cold methanol. The dried deposit weighed 26.8 gm. It was dissolved in a little hot water, and the filtered solution (charcoal) concentrated to incipient crystallization and allowed to cool. Recrystallized from alcohol, it formed feathery needles, m.p. 340° C., identified as potassium nitrate.

Isolation of Lignin

The original methanolic filtrate from which the potassium nitrate had been filtered was poured into four litres of hot water and the flocculent precipitate filtered with suction and washed with water. The light-brown, amorphous powder was dissolved in dilute caustic, the solution filtered and acidified to Congo red with dilute hydrochloric acid. A granular precipitate formed immediately and was washed by decantation with hot (80° C.) water. Filtered and dried, it amounted to 7.2 gm. and was found to consist of lignin.

Isolation of i-Inositol

A warm solution of basic lead acetate was poured into the dark-brown aqueous-methanol filtrate from which the lignin had been removed and the precipitate filtered off. After de-leading with hydrogen sulphide, and removal of the lead sulphide, the filtrate was concentrated under reduced pressure to a syrup which was boiled with methanol and again concentrated. A large proportion was then insoluble in absolute methanol. The soluble portion yielded an additional 4 gm. of potassium nitrate. A sample of the syrup failed to yield a crystalline osazone when treated with phenylhydrazine.

The brownish-yellow lead precipitate was suspended in water and de-leaded with hydrogen sulphide, the lead sulphide filtered off and the filtrate concentrated to a syrup under reduced pressure. This was dissolved in methanol and again evaporated to a syrup, the process being repeated several times. Finally, the syrup was dissolved in methanol (charcoal) and the solution allowed to stand at room temperature. It deposited small, stout, colorless crystals which produced the usual salmon color in Sherer's test for inositol. Recrystallized from boiling alcohol it melted at 224–225° C. Calcd. for $C_6H_6(OH)_6$: C, 40.00; H, 6.67%. Found: C, 39.70; H, 6.63%. The presence of *i*-inositol in wheat straw, therefore, is established.

Aqueous Extract

All efforts to isolate the coloring matter present in this liquor failed. Extraction with various solvents did not remove anything and even treatment with basic lead acetate yielded nothing but trifling amounts of intractable gums.

Dilute Caustic Soda Extract

After filtration the dark brown solution was acidified with dilute hydrochloric acid and the flocculent precipitate washed repeatedly with hot water

(80° C.) by decantation and on the filter. This precipitate, which consisted of lignin, yielded a brown amorphous powder (15 gm.) which could be methylated with dimethyl sulphate and 50% potassium hydroxide, yielding a methyl derivative no longer soluble in sodium hydroxide.

Acknowledgment

The author wishes to acknowledge his indebtedness to Mr. C. W. Davis, of these laboratories, who determined all the iodine numbers.

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MEASUREMENT OF THE HEATS OF WETTING OF CELLULOSE AND WOOD PULP[1]

By G. H. Argue[2] and O. Maass[3]

Abstract

The heats of wetting of dry cellulose and wood pulp have been measured for the first time. The influence of the initial water content of the cellulose on the heat of wetting has been determined. It is shown that when the water is left as a result of partial desorption a higher value is obtained. The heats of wetting of wood meal and different wood pulps have been determined. The heats of adsorption were calculated, and all the data are discussed from the point of view of the physical structure of cellulose. A few preliminary measurements of the heat of adsorption of sodium hydroxide were made. The experimental technique of using a rotating adiabatic calorimeter is shown to be admirably adapted for measurements of this kind.

Introduction

Information regarding the colloidal system cellulose-water is not only of scientific interest but of practical importance. Adsorption and desorption isothermals have been measured (13-15), and volume changes which take place on the addition of water to cellulose have been observed (3). From the data thus obtained inferences have been drawn as to the mechanism of water adsorption and the colloidal structure of cellulose. The purpose of the present investigation was to gain more information regarding the nature of water adsorption on cellulose by measuring the heat of wetting, and to correlate this with the conclusions drawn from the first-mentioned data. In this connection it was realized that it would be important to carry out measurements of the heat of wetting of cellulose pretreated in different ways.

There are few data available. The heats of wetting of several cellulose acetates have been indirectly determined by Newsome and Sheppard (9). Katz (5, 6) made direct measurements on the heat of wetting of one sample of cellulose. The accuracy of the technique developed in this laboratory (7) for the measurement of small heat changes opened the way for a thorough investigation of the heat of wetting of cellulose.

Apparatus

The apparatus employed in this work was essentially the same as that first used by Lipsett, Johnson and Maass in the determination of the heat of solution of sodium chloride in water, and in the measurement of the surface energy of solid sodium chloride (7, 8). It consists of a small rotating adiabatic calorimeter with a small inner silver box, the latter having a removable cover. The purified cellulose was placed in this, a definite weight of water was put in the silver calorimeter and the apparatus was assembled. Temperature control between the inner and outer calorimeter was maintained by the use of

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a multiple-junction, radiation thermocouple of copper-constantin. The temperature of the water bath was measured by means of a platinum thermometer with a Mueller bridge and a sensitive galvanometer. For details of the apparatus, its manipulation and calculation of results with the necessary corrections, the reader is referred to the original papers (7, 8).

Considerable trouble in removing the cover of the box containing the cellulose was experienced in the early experiments. The amount of vaseline that it was necessary to use in order to prevent water vapor from gaining access to the cellulose prevented the cover from falling off when rotation began. To obviate this the cover of the inner silver box was made of iron. This was silver plated to prevent rusting. A strong electromagnet of the horseshoe type was mounted in the outer water bath directly over the lid of the calorimeter. When temperature equilibrium had been attained and the initial temperature measured, a current was passed through the electromagnet and the rotation started simultaneously. The pull on the lid due to the magnetic field, added to that due to the rotation, dislodged the lid in spite of a thick layer of vaseline. Thereupon the magnet was immediately removed from the water bath, so that the heat generated by the electric current would not interfere with the adiabatic control.

Purification of Cellulose

The cellulose was prepared from pure cotton. This was placed in a flask and refluxed with a 1% sodium hydroxide solution for 12 hr. The liquor was poured off, the sample washed with distilled water and the procedure repeated seven times. The cellulose was then acidified with a 1% acetic acid solution and washed free of acid with distilled water. The excess water was pressed from the cotton and the purified sample was air dried for a day, then placed in an oven at 100° C. for two days and finally was transferred to a storage bottle. In this paper, cellulose prepared in this way will be designated as standard cellulose.

Procedure

An adequate amount of cellulose was used and the fibres were separated as much as possible. This was heated for $3\frac{1}{4}$ hr. in an electric oven maintained at $100 \pm 0.5°$ C. The sample was then transferred to the silver box while in the oven, and the lid was placed in position. Experiments showed that no moisture from the air was taken up by the cellulose during this operation. The weight of the sample after this treatment was considered as the dry weight of the cellulose, and all calculations are based on this degree of dryness. Approximately 50 cc. of water was placed in the calorimeter from a weight pipette, and the apparatus was assembled.

After thermal equilibrium had been attained the temperature was read for 10 to 15 min. and could be maintained constant to $\pm 0.0001°$ C. When the reaction began, the evolution of heat in the calorimeter was compensated for by the addition of hot water to the outer bath. The rotation was continued for 20 min., temperature equilibrium being maintained during this time.

The rotation of the calorimeter was stopped and the final temperature was measured for a period of time. In order to correct for the mechanical heat generated during the rotation, the calorimeter was rotated a second time and the temperature rise per minute was calculated and this correction applied to the resultant temperature.

In some experiments the initial water content of the sample was brought to a definite value. Here the dry weight of the cellulose was found by first weighing the sample in a small glass weighing bottle under conditions as described above. The cellulose was transferred to the silver box and allowed to absorb moisture from an atmosphere containing a definite amount of water vapor. The sample was then weighed in the closed silver box. The weights of cellulose and water were converted to those *in vacuo*, and the weight of water per gram of dry cellulose was calculated.

In the experiments which are labelled "Desorption", the dry weight of cellulose was determined as described above. The sample was saturated with water, placed in the open silver box and heated in a vacuum desiccator over phosphorus pentoxide to 50° C. The time for this treatment was adjusted so as to leave a desired amount of moisture on the sample. The lid was replaced and the exact amount of water determined by weight.

The heat constant for the silver calorimeter was determined by measuring the heat of solution of sodium chloride in water and making use of the data obtained by Lipsett, Johnson and Maass (7, 8). The specific heats of the component parts being known, a calculation of the heat capacity of the calorimeter was made in this way and found to agree with that obtained by direct measurement. The specific heat of cellulose was considered to be 0.32 cal. per gram (4).

The temperature change of the calorimeter could be measured to within 0.0002° C., and as the temperature change varied from 0.2 to 0.08° C., depending on the initial amount of water adsorbed, an accuracy of from 0.1 to 0.5% was possible in the heat measurements. The average weight of the cellulose sample taken was 2 gm., and since weighing could be carried out to 0.1 mg. the weight of water adsorbed could be estimated to 0.5% for a sample having an initial water content of 1%, and more accurately for the initial water adsorptions.

Results

The initial values found for the heat of wetting varied considerably more than could be accounted for by the calculated limit of error. These early experiments were carried out with cellulose samples that had not been treated in a uniform manner, both with regard to purification and uniformity of water adsorption. The magnitude of the heat measured depends on this and on other influences to which the cellulose had been subjected previous to the measurement. As soon as this was realized the exact procedure of pretreatment as outlined above was followed and duplicate checks could be obtained. The data shown in Table I for standard cellulose are the averages of approxi-

mately 50 determinations made with samples which had been subjected to identical chemical and heat treatment before the measurement was made. These results are shown graphically in Fig. 1.

The range of concentration of adsorbed water covered for the heat of wetting was 0.0 to 0.07 gm. of water per gram of cellulose. Of the various determinations, those with dry cellulose could be made most readily and afforded the best checks. With concentrations of adsorbed water greater than 0.07 gm. it was difficult to bring about uniform adsorption on the cellulose, and, as the results beyond this concentration are of lesser interest, no attempt was made to modify the procedure in order to make these measurements possible.

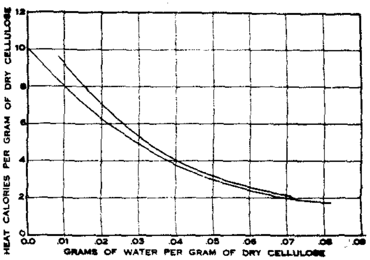

FIG. 1. *Heat of wetting of cellulose containing adsorbed water and that of cellulose containing water left as a result of partial desorption.*

TABLE I

EXPERIMENTAL RESULTS ON THE HEAT OF WETTING OF CELLULOSE CONTAINING ADSORBED WATER AND THAT OF CELLULOSE CONTAINING WATER LEFT AS A RESULT OF PARTIAL DESORPTION

Adsorption		Desorption		Adsorption		Desorption	
Water content, gm.	Heat of wetting, cal.	Water content, gm.	Heat of wetting, cal.	Water content, gm.	Heat of wetting, cal.	Water content, gm.	Heat of wetting, cal.
0.0	10.16			0.04	3.78	0.04	4.04
0.005	9.0			0.05	2.98	0.05	3.20
0.01	8.0	0.01	9.29	0.06	2.39	0.06	2.64
0.02	6.27	0.02	7.1	0.07	1.99	0.07	2.17
0.03	4.9	0.03	5.32	0.08	1.78		

NOTE:—*Water content is the weight of water per gram of dry cellulose present before measurement of the heats of adsorption and desorption. Heat of wetting is the heat of wetting per gram of dry cellulose in the sample.*

The desorption range was from 0.015 to 0.07 gm. of water per gram of dry cellulose. At lower concentrations the results were not consistent and cannot be expected to be so, as will be explained later.

From the data in Table I and its graphic presentation a number of calculations can be made. Since the heat of wetting of dry cellulose is 10.16 cal. per gram of cellulose, the heat of adsorption H_1 given out when x grams of water is added to 1 gm. of cellulose is obtained by subtracting the heat

of wetting of 1 gm. of cellulose containing x grams of water from 10.16 cal. These data are given in Columns 1 and 2 of Table II.

These results can be put in another way: $1/x$ is the number of grams of cellulose on which 1 gm. of water will give an amount of adsorbed water equal to the concentration given in Column 1. H_1/x will then represent the amount of heat given out when 1 gm. of water is added to $1/x$ grams of cellulose. The fifth column of Table II shows the heat of adsorption of 1 gm. of water vapor on cellulose containing per gram the amounts of water shown in Column 1, the weight of cellulose being such that the addition of this water does not change the amount of adsorbed water. This heat may be calculated as follows:— if dx gm. of water, added to 1 gm. of cellulose containing x grams of water, gives out dH_1 calories, 1 gm. of water on $1/dx$ gm. of cellulose will give out dH_1/dx calories on cellulose containing x grams of water per gram of cellulose. dH_1/dx is the heat of adsorption of 1 gm. of liquid water on an infinite amount of cellulose at this water concentration. The values of dH_1/dx can be obtained by differentiating the equation

TABLE II

CALCULATIONS OF THE HEAT OF ADSORPTION OF WATER VAPOR ON CELLULOSE

x, gm.	H_1, cal.	$1/x$, gm.	H_1/x, cal.	H_2, cal.
0.0	0			
0.005	1.16	200	232	
0.01	2.16	100	216	775
0.02	3.89	50	194.5	740
0.03	5.26	33.4	175.3	697.9
0.04	6.38	25	159.5	668.9
0.05	7.18	20	143.6	651.6
0.06	7.77	16.7	129.5	639.9
0.07	8.17	14.3	116.7	631.7
0.08	8.38	12.5	104.8	625.5

NOTE:—*x is the weight of water adsorbed per gram of dry cellulose, H_1 the heat of adsorption of x gm. of water on 1 gm. of cellulose, $1/x$ the weight of dry cellulose required per gram of water to give adsorption x, H_1/x the heat of adsorption liberated when 1 gm. of water is added to $1/x$ gm. of cellulose, and H_2 the heat of adsorption of 1 gm. of water vapor on an infinite amount of cellulose containing x gm. of adsorbed water.*

$$H_1 = 8.01 \log x + 17.49,$$

which accurately represents the data for the range 0.02–0.07 gm. of water per gram of cellulose. For values less than 0.02 gm. recourse was had to graphic estimation of the tangent to a curve representing the data of Columns 1 and 2. To dH_1/dx is added 582 cal., the heat of condensation of water at 25° C.

In Fig. 2 is shown the heat of adsorption of water vapor on cellulose containing various amounts of adsorbed water. The data in Column 4 of Table II plus 582 cal. when extrapolated to zero concentration should give the same values as those of the heat of adsorption of water vapor on dry cellulose. The upper curve representing these data shows that this is the case, a value 250 cal. greater than the heat of condensation of water vapor being obtained for the heat of adsorption of 1 gm. of water under these conditions. It is seen from the figure that the heat of adsorption falls rapidly with increasing concentration of adsorbed water, and will eventually become the same as

the value of the heat of *condensation of water vapor*. The greatest change takes place during the adsorption of the first 0.04 gm. of water per gram of cellulose.

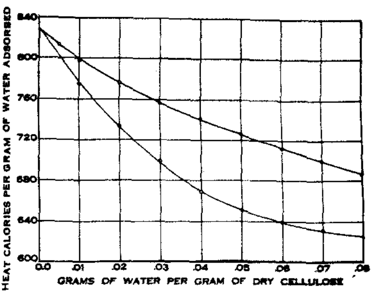

FIG. 2. *Heat of adsorption in calories of 1 gm. of water vapor on cellulose containing various amounts adsorbed water.*

Discussion

For clarity in discussion of the experimental results it is necessary to present the authors' views regarding the physical structure of cellulose. Cellulose consists of long chains made up of a hundred or more glucose units. A number of these chains (approximately 40 or more) are symmetrically connected together in a bundle to form sub-microscopic crystals or micelles. The fibre of cellulose is composed of these micelles which are in contact with one another over a considerable part of their surface. Where contact occurs, crystal forces similar to those holding the chains in the micelle come into play. The remaining micelle surface may conveniently be called the inner free surface of the cellulose.

Gas molecules can penetrate into the fibre, reaching this free surface, the cellulose crystals themselves remaining impenetrable. Adsorption of gases can take place on this free surface, and the first portion of water vapor to enter a dried fibre is adsorbed in this manner. The water vapor entering subsequent to the saturation of this surface will condense and fill the intermicellar spaces. The amount of water held by the cellulose for a given vapor pressure depends on whether water has been added to the dried cellulose or abstracted from cellulose saturated with water vapor. In other words, adsorption is followed by hysteresis when desorption takes place.

Because of this and the form of the adsorption isotherm it follows that the water also increases the inner surface in the sense that contact areas between micelles are disrupted, forces between cellulose and water replacing those of cellulose to cellulose. Since cellulose does not disperse into its micelles when placed in liquid water, the cellulose-to-cellulose attraction must be greater than the cellulose-to-water. Hence the cellulose-to-cellulose bonds that are disrupted by the water are those which are under a particular strain due to the manner in which the micelles are arranged in the fibre. Thus, after the water vapor required for saturation of the originally present inner free surface has been adsorbed, subsequent addition of water vapor creates more surface and the lowering of the vapor pressure is greater than need be accounted for by the curvature of the liquid water in the intermicellar spaces. The form of the sorption curves is in agreement with this. Furthermore, on desorp-

tion the first water removed is that in the intermicellar spaces. Water held on the freshly created surface will require a lower vapor pressure to be removed than would be the case if the water were in the intermicellar spaces. When this water is removed the cellulose surfaces can join up again.

The foregoing hypothesis of the mechanism of water adsorption is in many respects similar to that proposed by Urquhart (12). The idea of strains helping to disrupt micelle surfaces from one another was first put forward by Campbell to correlate the adsorption isotherms of water, and propyl and methyl alcohols (1, 2).

In addition to the above it is reasonable to suppose that the inner free surface is not uniform in regard to its absorbing power. For instance, the ends of the molecular chains will be fundamentally different from the sides, and presumably present a greater amount of free valency for adsorption purposes. Over all the surface there will be active groups affording regions on which there will be pronounced adsorption. From such centres the tendency to adsorption gradually falls off.

When liquid water is brought into contact with cellulose, water is adsorbed, giving rise to an evolution of heat; at the same time the surface is increased. This requires energy for the splitting of the cellulose-to-cellulose bonds between micelles. The greater the amount of water initially present in the cellulose the smaller are both effects. This explains the data obtained from the "desorption" and "adsorption" experiments and shown graphically in Fig. 1. Cellulose from which a portion of the water has been desorbed has a larger surface than cellulose containing the same amount of water added to a dry sample. When liquid water is brought into contact with these two samples both will have the same final surface area. Hence in the former case less energy is required for surface formation, and consequently the heat of wetting is greater. When water is desorbed from wet cellulose to such an extent that there is less than 0.02 gm. of water per gram of dry sample, cellulose reverts to the dry state, and the extent to which it does is dependent on the rate of water removal and experimental conditions. Thus, reproducible experimental values could not be obtained in this region, as was pointed out in the experimental section. When 1 gm. of water vapor is adsorbed on an infinite surface of dry cellulose, 250 cal. more than the heat of condensation is given out. On a sample prepared differently this limiting value was found to be 280 cal. It would appear therefore that these two cellulose surfaces differ from each other, and this is in agreement with the hypothesis stated above. If the adsorption were not uniform in intensity on the different parts of the molecular chains, samples having different chain lengths and different micelle distributions would give rise to differences in the average intensity with which sorption takes place on their surfaces.

The heat of adsorption per gram of water falls rapidly with an increase in the moisture content of the cellulose, reaching one-fifth of the value for dry cellulose when the cellulose contains one-third of the quantity of water required for saturation of the fibre—in this case 0.07 gm. water per gram of

cellulose. At this stage the relative vapor pressure is 50%, a reduction in vapor pressure which would be given by capillaries having a diameter corresponding to approximately ten water molecules. The general shape of the curve for heat of adsorption will be discussed in another paper, in correlation with dielectric constant determinations on cellulose containing various amounts of adsorbed water.

When the heat of adsorption of 1 gm. of water per infinite amount of cellulose is calculated from the desorption experiments, it is found that the the heat of adsorption is definitely greater when adsorption takes place on a cellulose sample from which a portion of the water has been desorbed. Thus when 0.02 gm. of water per gram of cellulose has been left after desorption it is 773.5 cal. as compared to 733 cal. When the water content is 0.03 gm. of water per gram of cellulose the values are 709.7 and 687.9 cal. This shows that a larger surface is available on cellulose from which water has been desorbed.

In a recent paper Stamm (10) has calculated the heats of adsorption from vapor pressure isothermals. It is of interest to do the reverse and calculate the isothermals from the heats of adsorption. Data of Urquhart and Williams (13-15) are taken for this purpose. The 25 and 100° C. isothermals are takeh and the latter calculated by means of the equation

$$H = \frac{1.98 \times 298 \times 373 \times 2.3}{18\ (373-298)} (\log h_2 - \log h_1),$$

where H is the heat of adsorption given by the measurements of the heat of wetting in Table II, and h_1 and h_2 are the relative humidities.

In Table III the first column shows the moisture content, the second and third columns the experimental data of Urquhart and Williams and the fourth column the humidity at 100° C., calculated from our data.

TABLE III

CALCULATION OF THE ISOTHERMAL VAPOR PRESSURE FROM HEAT-OF-WETTING DATA

Moisture content, gm. water/gm. dry cellulose	h_1 (25° C.)	h_2 (100° C.)	h_2 (100° C.) Calcd.
0.02	0.10	0.242	0.253
0.03	0.222	0.390	0.449
0.04	0.340	0.550	0.580
0.05	0.45	0.656	0.689
0.06	0.57	0.745	0.814

It is seen that the agreement over the range 0.02–0.05 gm. of water per gram of cellulose is good. Below this range the calculated values are too high and above this range they are too small. The assumption made in the calculation, that the heat of adsorption is independent of the temperature, cannot be strictly true. It is also apparent that the heat of wetting of cellulose containing less than 0.02 gm. of water cannot be calculated with any accuracy from the isothermals. The variation of the heat of wetting with the water content is greatest here, and the data for the amount of adsorption are the least accurate in this region. That the heat of wetting of dry cellulose is of particular interest will be made apparent in the following section.

Heat of Wetting and the Physical Structure of Cellulose

The heat of wetting of wood pulp is considerably larger than that of cotton cellulose. Nine samples obtained from different cooking processes were examined and the heat of wetting was found to lie between 12.3 and 13.3 cal. The specific viscosities of 1% solutions of these pulps in cuprammonium were measured, as also was the viscosity of a sample of cotton pulp whose heat of wetting was found to be 10.5 cal. Assuming Staudinger's theory, that these viscosities show the length of molecular cellulose chains, to be correct, no relation was found between the length of chain as given by this viscosity and the heat of wetting.

The viscosities of xanthate solutions of these pulps, prepared under identical manufacturing conditions, were measured by the falling sphere method. The pulp having the largest heat of wetting had the smallest viscosity in xanthate solution, 5.1 poises, whereas the cotton pulp had the largest viscosity, 10.7 poises. Within the accuracy of measurement, the viscosities in xanthate solution and the heats of wetting of the other samples followed the same sequence.

When the cellulose is converted into viscose, the chains are broken down during the shredding and aging periods, and at the same time secondary forces between the micelles are broken up owing to the action of caustic soda, atmospheric oxygen and carbon disulphide. The viscosity of viscose indicates the extent to which the chains can be separated from one another. A relatively low viscosity means a breaking up into smaller particles. This is more likely to happen the greater the internal surface. The greater heat of wetting corroborates this. The extent of the internal surface is therefore dependent more on the number of chains to the micelle than on the length of the chains. This is to be expected, for the length of a micelle is many times as great its diameter. The heat of wetting may prove to be of interest in predicting the extent of viscose dispersion, and experiments in this direction are contemplated.

TABLE IV

HEAT OF WETTING PER GRAM OF DRY CELLULOSIC MATERIALS (CAL.)

Standard cellulose	10.16
Cotton pulp	10.54
Kraft pulp	13.08
Bleached sulphite pulp:—	
Beating time, min. 0	12.68
Beating time, min. 10	13.01
Beating time, min. 20	13.48
Beating time, min. 30	13.61
Beating time, min. 40	13.92
Wood meal:—	
Sapwood, −100 mesh	19.78
−40 +100 mesh	19.26
Heartwood, −100 mesh	18.41
−40 +100 mesh	18.13

In Table IV the heats of wetting of a number of cellulosic materials are shown in the second column. In the first column the nature and, if necessary, the special condition of the material are indicated.

It is of interest to note that apparently the heat of wetting of a pulp increases only slightly with beating. In the beater, the outside area of the pulp particles is increased 100-fold, but as the internal area of the smallest particle formed is probably thousands of times its external area, the total area available for

wetting is increased only to an insignificant extent by the increased subdivision. What increase in the heat of wetting has been found must therefore be due to an increase in the internal area during beating. Such an increase is to be expected, as the mechanical stresses to which the fibres are subjected during the beating may well bring about a loosening of some micellar bonds.

Campbell (1, 2) has shown that the amount of adsorption of water does not increase during beating to the extent that would be expected on the basis of the "hydration theory of beating" (11), and he showed that this theory was untenable. His recent accurate determinations show an average increase of about 4% in adsorption for pulp beaten to the same extent as the sample mentioned in Table IV. The heats of wetting given above show a somewhat greater percentage increase. In a number of other samples of beaten pulp the increase in the heat of wetting was found to vary from 2 to 10% for 40 min. of beating, but since the beating conditions were not uniform these are not tabulated and mention of them is made simply to show the order of magnitude of the increase in the heat of wetting.

The heat of wetting of wood meal is much greater than that of cellulose, as the figures show; that of sapwood is slightly greater than that of heartwood, and increased subdivision apparently causes a small increase in the heat of wetting.

The adsorption of water vapor on standard cellulose, bleached sulphite pulp and wood meal has been found to be 0.060, 0.070 and 0.10 gm. of water vapor per gram of dry material respectively at 50% relative humidity. The proportionality factor to the data on the heat of wetting is 1.70, 1.8 and 1.84 respectively. As might be expected the adsorption is practically proportional to the heat of wetting. This is in agreement with the fact that the heat of wetting of cellulose from which a portion of the water has been desorbed is greater than that of cellulose containing an equal amount of water due to adsorption as shown previously.

The apparatus described is admirably adapted for other investigations of a nature similar to the foregoing. Some measurements have also been made on the heat of wetting of cellulose in sodium hydroxide solutions. Standard cellulose containing approximately 5% of water showed a heat of wetting of 12.6 cal. per gram of dry cellulose in a 2% solution of sodium hydroxide. In more concentrated solutions the heat of wetting was greater, being 21.5 and 13.3 cal. for dry samples in 16 and 8% solutions of sodium hydroxide. The measurement of the heat of wetting of cellulose in alcohols is under way, and in a number of preliminary experiments it has been found that the heat of wetting with 95% methyl alcohol is 6.2 cal. per gram of cellulose.

It will be interesting to make measurements of heats of wetting with alcohol solutions containing varying amounts of water. It is hoped that these will throw further light on the validity of the interpretation of the experimental results which have tentatively been made by the authors.

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INFLUENCE OF WILD AND CULTIVATED PLANTS ON THE MULTIPLICATION, SURVIVAL AND SPREAD OF CEREAL FOOT-ROTTING FUNGI IN THE SOIL[1]

By G. W. Padwick[2]

Abstract

Using the severity of infection of wheat seedlings as a measure of soil infestation, it is shown that susceptible grasses such as *Agropyron tenerum*, *A. cristatum*, *A. repens* and *Bromus inermis* encourage the multiplication and survival of inoculum of the take-all fungus *Ophiobolus graminis* in both sterilized and unsterilized soil. The same grasses also aided the survival of *Helminthosporium sativum* in sterilized soil. In these experiments, however, only one, namely *B. inermis*, appeared to favor the survival of *Fusarium graminearum*.

The fungus *O. graminis*, which failed to spread laterally to any appreciable extent in bare, unsterilized black loam soil of the Edmonton district of Alberta, was able to do so when such soil was occupied by living, susceptible plants.

Introduction

The present study was undertaken primarily with the object of ascertaining in what ways the growth of higher plants may influence the subterranean behavior of the fungi causing foot rots of wheat. Three foot-rotting organisms were selected for study, namely *Ophiobolus graminis* Sacc., *Helminthosporium sativum* P.K.B. and *Fusarium graminearum* Schwabe. At the beginning of the investigation it was realized that several effects of the growth of higher plants on such fungi may be possible. Of these, five are suggested as having an important influence.

(i) By serving as selective media for the parasites, to the exclusion of the saprophytes which normally by their competitive action help to keep the parasites in check, higher plants of susceptible species may permit an abundance of inoculum to be built up.

(ii) Susceptible plants may be an important means by which pathogenic organisms persist in field soils.

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(iii) The pathogenes may spread through the soil more rapidly on, or in, the roots of susceptible plants than in bare soil where the spread is checked by competing organisms.

(iv) Higher plants, either living or dead, may be the chief means of overwintering of the pathogenes in the soil.

(v) The living underground parts of plants may secrete substances capable of stimulating or of inhibiting the growth of organisms in the soil, and the presence of dead tissue of such plants may serve the same purpose.

The first three aspects of the problem have been studied experimentally.

Review of Literature

A disease somewhat analogous to cereal foot and root rots is the cotton root-rot disease, which causes serious losses in the United States of America. Taubenhaus and Killough (31) in 1923 stated their belief that clean culture involving the destruction of the winter carriers of the causal fungus, *Phymatotrichum omnivorum*, was of value in helping to control the cotton root-rot disease, while rotations involving only partial freedom from susceptible hosts were not effective. Taubenhaus, Dana and Wolff (30) in 1929 concluded that it was impossible to control cotton root rot without destroying susceptible perennial weeds. McNamara and Hooton (18) found a one-year fallow insufficient, a two-year fallow or a one-year fallow in combination with a rotation system being necessary for control of cotton root rot.

In the case of wheat, the effect of rotations on root-rot development has been studied by a great many workers. This work has recently been so thoroughly reviewed by Broadfoot (3) that it is unnecessary to go into details here. Special attention however may be called to the names of Henry (11), Sewell and Melchers (28), Greaney and Bailey (10), Russell (23, 24) and Sanford (26). Similarly, the literature on the host range of the three fungi has been reviewed by Padwick and Henry (20).

All the organisms studied here are able to carry on a saprophytic existence in some soils, but the extent to which they do so under natural conditions is not easily determined.

Kirby (16) found that soil infested with *Ophiobolus graminis*, when screened and kept indoors for eight months, lost its ability to cause take-all of wheat, while bits of straw containing perithecia were able to cause severe infection when similarly treated. Davis (5) and Russell (22), however, have found this fungus to persist in bare soil for considerable periods. Russell for instance (25) found that *O. graminis* can remain viable in bare soil for at least two years and then infect wheat seeded in the soil, but that there was a marked decrease with time in the aggressiveness shown by the fungus.

Henry (12) found strong inhibition of *H. sativum* produced by the addition to sterilized soil of small quantities of unsterilized black loam soil at the same time that inoculum of the pathogene was added, and suggested that under natural conditions micro-organisms comprising the natural soil flora have a

marked suppressive action on foot-rotting organisms. The same writer (13) found that spores of *H. sativum* occur rarely if ever in field soils, owing to the inhibitive influence of other micro-organisms. He later (14) found in studies with *O. graminis* that this inhibitive action of the normal soil flora varies with temperature. Attacking the problem in a different way, Sanford and Broadfoot (27) and Broadfoot (2) studied the mode of action of other soil inhabiting micro-organisms on the virulence of *O. graminis*, finding numerous inhibitors and a few compatible organisms.

Garrett (9) utilized the rate at which hyphae of *Ophiobolus graminis* grew externally along seminal roots of wheat as an indication of the suitability of the environmental conditions of the soil for infection of wheat. Soil saprophytes were found to impede the progress of the hyphae.

The difficulty of isolating *Ophiobolus graminis* from the soil and from infected plants, its weak saprophytic growth, and the inconspicuous nature of the symptoms of the disease it causes when in the early stage, have made a knowledge of the distribution and progress of the organism in the soil under field conditions difficult to obtain. Russell (23) was unable to demonstrate the spread of take-all from inoculated seedlings in the centre of eight-inch crocks to seedlings sown closely around them, the neighboring plants reaching maturity without showing symptoms. Fellows (7) has found the organism present to a depth of at least ten inches in infested soils in Kansas, but it is possible that the organism may have been carried to this depth by deep plowing. It seems to be the popular belief that the circular patches of diseased wheat plants in fields are the result of the radial growth of *O. graminis* from the centre of infection. Similar circular spots in *A. repens* have been observed (20) to be very marked in a field which remained uncultivated for several years and was supporting a thick stand of this weed.

Influence of Weeds and Grasses on the Multiplication and Survival of Foot-rotting Organisms in the Soil

Experimental Methods

Five species of higher plants of economic importance in the prairie provinces of Canada were selected in order to determine their effect on the survival of *O. graminis*, *H. sativum* and *F. graminearum* in the soil and their role in increasing the amount of inoculum for infecting wheat. Four of the plants studied were gramineous species, namely *Agropyron tenerum* (slender wheat grass) and *A. cristatum* (crested wheat grass), which are cultivated as forage crops in western Canada; *A. repens* (couch grass), a common weed in many parts, and *Bromus inermis* (brome grass), which is grown extensively for forage purposes. All these grasses are perennials. The fifth species was a dicotyledonous plant, *Neslia paniculata* (ball mustard), an annual weed.

The method adopted was to apply inoculum of the various organisms to pots of soil, together with seed of the species to be studied, and after a period of growth varying from five to eight weeks to seed the pots to wheat (*Triticum*

vulgare), which served as an indicator of the survival of the fungus in the soil and of its ability to reinfect wheat in planted soil as compared with controls in unplanted soil. Some experiments were run concurrently, using the same controls; others were run separately and separate controls had to be used. In certain instances, owing to unsuitable temperatures obtained in the greenhouse while the experiments were being conducted, some series, together with their controls, had to be repeated in their entirety.

The inoculum was prepared by growing the organisms in Erlenmeyer flasks containing 50 gm. of black loam soil which was moistened with 28 cc. of tap water and sterilized by autoclaving. The fungus was allowed to grow in the flasks for 17 days at room temperature. Six-inch pots were two-thirds filled with black loam soil and half of the pots were then sterilized in the autoclave for four hours. The entire contents of a flask was then added to each pot. Seeds of the plant species to be studied were then sown in the pots and covered with soil. Ten replicates of each treatment were seeded, so that every species under investigation was grown in ten pots with each of the three fungi in sterilized soil, and ten pots in unsterilized soil. Other pots were filled and had fungi added in a similar manner, but were not seeded, and these were used for comparison with the seeded pots. All the pots were then placed in the greenhouse at approximately 25° C., and the plants were allowed to grow for five to eight weeks. The top growth of the plants was then cut off and each pot was seeded with 25 seeds of Marquis wheat, which had previously been treated with hot water in order to kill as far as possible any pathogenic fungi which might be present in or on the seeds. After three weeks the wheat seedlings were removed and were assigned infection ratings from 0 to 5 according to the degree of rotting of stems and roots. These were averaged for each pot and series of pots and expressed as percentages of the greatest possible degree of rotting.

Experimental Results

Considering the three organisms separately, (Table I) it is seen that in the case of *Ophiobolus graminis* there was in each instance a very marked and significant increase in the degree of infection of wheat following grasses in both sterilized and unsterilized soil. In fact, in the absence of weeds, there was no trace of infection when unsterilized soil was used. It is also clear that *Neslia paniculata* effected no significant difference in the amount of inoculum as measured by infection of wheat.

With *Helminthosporium sativum* the most striking results were found on sterilized soil, where, following all the grasses, with the exception perhaps of *Bromus inermis*, there was a very marked increase of infection. On unsterilized soil the grasses effected only a small increase, while *Neslia paniculata* effected none.

There was no significant increase of *Fusarium graminearum* foot rot as a result of the growth of species of *Agropyron*, nor was any increase caused by growth of *Neslia paniculata*. Wheat after *Bromus inermis* in unsterilized soil, however, showed a marked increase of *Fusarium* foot rot. There was

TABLE I

INFECTION OF WHEAT WITH *Ophiobolus graminis*, *Helminthosporium sativum* AND *Fusarium graminearum* FOLLOWING CERTAIN WEEDS AND GRASSES

Organism	Soil	Presence of grass or weed	*Agropyron tenerum*		*Agropyron cristatum*		*Agropyron repens*		*Bromus inermis*		*Neslia paniculata*	
			Degree of infection of wheat plants, %	Probability[1]	Degree of infection of wheat plants, %	Probability[1]	Degree of infection of wheat plants, %	Probability[1]	Degree of infection of wheat plants, %	Probability[1]	Degree of infection of wheat plants, %	Probability[1]
Ophiobolus graminis	Unsterilized	Absent	0		0		0		0		0	
	Unsterilized	Present	11.3	+ +	1.8	+	5.2	+ +	9.9	+ +	0	—
	Sterilized	Absent	0.4		0.4		0.4		0.4		0	
	Sterilized	Present	13.0	+ +	5.8	+ +	6.7	+ +	15.9	+ +	0	—
Helminthosporium sativum	Unsterilized	Absent	9.4		9.4		9.4		5.6		5.0	
	Unsterilized	Present	14.5	0	15.3	0	15.4	+	19.1	+ +	4.9	0
	Sterilized	Absent	3.3		3.3		3.3		31.8		30.7	
	Sterilized	Present	14.7	+ +	9.8	+ +	9.6	+ +	51.0	+ +	30.3	0
Fusarium graminearum	Unsterilized	Absent	17.5		17.5		17.5		3.6		7.2	
	Unsterilized	Present	23.0	0	22.2	0	20.0	0	27.1	+ +	7.8	0
	Sterilized	Absent	30.6		30.6		30.6		44.9		31.4	
	Sterilized	Present	26.1	0	20.2	0	32.5	0	41.1	0	21.4	0

[1] *Probability refers to the odds according to the method of Student. Odds below 30 : 1 are considered to mean that the difference is not significant (0); odds of 30 : 1 to 200 : 1 mean a significant difference (+); and above 200 : 1, very significant (+ +).*

no increase of infection in sterilized soil, but in view of the fact that in bare sterilized soil the infection reached 44.9%, much increase would perhaps not be expected.

The results in general show a close agreement with what was to be expected from results of experiments previously reported (20). In those experiments it was found that all the species of *Agropyron* studied were severely damaged by *O. graminis*, as also was *Bromus inermis*. The present results show that, in bare unsterilized soil, the organism, under the conditions existing, seemed to have become incapable of attacking wheat, while in soil planted to *A. tenerum*, *A. cristatum*, *A. repens* and *B. inermis* considerable infection of the wheat occurred. In all cases a marked increase occurred in the degree of infection of wheat with *O. graminis* in unsterilized soil after susceptible grasses, indicating a tendency for the organism to accumulate in soil planted to these grasses.

In the previous experiment all the four grasses studied were found susceptible to *H. sativum*, and all have served in the present experiment to increase the amount of inoculum of this organism in sterilized soil. The amounts of increase in unsterilized soil were not very significant. It was previously found that none of the four grasses appeared to be damaged by *H. sativum* in unsterilized soil to the same extent as they were by *O. graminis*, and for this reason it was only to be expected that the grasses would play a somewhat smaller role in carrying over the organism and serving as a source of inoculum for the succeeding wheat. *B. inermis* was the only grass of the four studied which in the previous experiments was infected and damaged by *F. graminearum* in unsterilized soil, and it is the only grass which in these experiments served to increase the amount of inoculum of this organism in unsterilized soil. In no instance did *Neslia paniculata* serve to aid in the survival or increase of any wheat foot-rotting pathogenes in the soil.

These results support the view previously expressed that the mere fact that a plant is susceptible to attack by wheat foot-rotting pathogenes under the unusual conditions of experimental inoculation, especially in sterilized soil, does not give an adequate indication of the role which it may play in the problem of foot rot in wheat. It is necessary to obtain an indication of the relative amount of damage done to these susceptible plants, in unsterilized as well as sterilized soil, before a reliable estimate of their importance in the problem may be made.

The Effect of Grasses on the Horizontal Spreading of Foot-rotting Fungi in Soil

Experimental Methods

Improved technique, developed by Mr. F. R. Davies in this laboratory, in isolating *Ophiobolus graminis* from plant tissues has made possible a study of the conditions under which this organism survives and spreads. Extensive experiments were outlined to determine whether this organism, and also

Fusarium graminearum, are able to spread progressively through unsterilized soil, both when bare and when planted to various grasses and to wheat.

Owing to the extensiveness of the experiment and the large amount of isolation work involved, the experiments were conducted with the two organisms at different times, first with *F. graminearum* (in the summer of 1932) and then with *O. graminis* (in the winter of 1932-33). There were, however, no essential differences in the methods adopted. On June 28, 15 flat wooden boxes, 25 × 16 × 3½ in., were filled to a depth of 2½ in. with unsterilized black loam soil, obtained from land kept bare for five years. Three boxes were seeded with wheat, three with *Agropyron tenerum* and three with *A. repens*. About 200 seeds were sown in each box. The remaining six boxes were not seeded. All were placed in the greenhouse at a soil temperature of about 20° C. and kept watered. On July 29, after the plants had become well established, a narrow trench was dug to the full depth of the soil, two inches from one end and across the full width of each box. In this was placed inoculum of *F. graminearum*, prepared by growing the fungus for 17 days in Erlenmeyer flasks each containing 45 gm. of moist sterilized soil plus 5 gm. of cornmeal. The contents of two flasks were used for each box. Three of the unplanted boxes were treated in the same manner, while in the trenches of the remaining three was placed a similar quantity of sterilized soil and cornmeal which, however, had no fungus growing on it. Thus it was possible to compare the spread of the organism in the presence of the two grasses, in the presence of wheat, and in unplanted soil. The purpose of the uninoculated boxes was to check against the natural occurrence of the fungus in the soil, which would at once render the results unreliable. On September 7, after removal of the top growth, Marquis wheat, which had been treated with hot water to reduce the seed-borne foot-rotting fungi to a minimum, was seeded in rows across the box along the original trench, and in rows parallel to it and two inches apart. Twelve rows were seeded in all. Twenty seeds were placed in each row, a small hole being made for each seed with a wooden meat skewer, a new skewer being used for each row. On September 27, the wheat plants from one box under each treatment were dug up and measured, the degree of infection of each plant was recorded, and the underground parts of plants in all rows showing any trace of foot rot were removed. These underground parts were surface sterilized by dipping for 1½ min. in mercuric chloride (1 gm. in a litre) and washing in 75% alcohol. The second group of boxes was harvested and treated similarly on October 1; and the third replicate on October 6.

A few slight modifications were made for *O. graminis*. As stated previously, the experiment was conducted during the winter, being commenced November 1. Inoculum of *O. graminis* (strain 108, obtained from *Agropyron repens*) was added November 29. In order to minimize washing of inoculum over the soil in the boxes, the boxes were tilted slightly, so that if the organism were carried at all by the water it would be in the opposite direction to that in which the rate of spread was to be determined. Only ten rows of wheat

TABLE II

RESULTS OF EXPERIMENTS ON THE SPREAD OF *Ophiobolus graminis* IN PLANTED AND UNPLANTED SOILS

Soil treatment	Distance from original place of application of inoculum, in.	Average length of wheat plants, cm.	Average degree of infection of wheat plants, %	Results of re-isolation trials of *O. graminis*
Soil left bare. Soil + cornmeal with no organism was placed at one end of the box. Included to check against natural occurrence of *O. graminis* in the soil.	0	26.0	0	−
	2	27.3	0	−
	4	23.2	0	−
	6	23.4	0	−
	8	23.8	0	−
	10	24.3	0	−
	12	21.8	0	−
	14	23.0	0	−
Soil left bare. *O. graminis* placed at one end of the box.	0	23.5	14.4	+
	2	24.1	1.1	+
	4	24.0	0	−
	6	23.8	0	−
	8	25.4	0	−
	10	23.2	0	−
	12	22.1	0	−
	14	23.7	0	−
Soil seeded to wheat. *O. graminis* placed at one end of the box.	0	28.8	14.1	+
	2	24.0	29.3	+
	4	20.0	29.4	+
	6	19.3	15.4	+
	8	22.0	7.7	+
	10	21.1	0.6	−
	12	21.5	0	−
	14	21.6	0	−
Soil seeded to *A. tenerum*. *O. graminis* placed at one end of the box.	0	25.6	9.7	+
	2	21.6	24.7	+
	4	21.2	10.7	+
	6	18.8	8.8	+
	8	19.1	7.2	+
	10	17.9	0.7	+
	12	18.9	0.6	+
	14	18.8	0	−
Soil seeded to *A. repens*. *O. graminis* placed at one end of the box.	0	26.3	8.6	+
	2	26.8	18.9	+
	4	21.2	13.2	+
	6	20.8	1.0	−
	8	20.2	0	−
	10	21.0	0	−
	12	20.6	0	−
	14	17.8	0	−

were seeded, on January 20, 52 days after adding the inoculum to the end of the box. All three replicates were harvested on February 14. In sterilizing for re-isolation, silver nitrate was used instead of mercuric chloride. Small portions of roots were dipped in 1% silver nitrate solution for one minute, and the silver nitrate was then precipitated with concentrated sodium chloride solution, after which the portions were plated on potato-dextrose agar in Petri dishes.

TABLE III

RESULTS OF EXPERIMENTS ON THE SPREAD OF *Fusarium graminearum* IN PLANTED AND UNPLANTED SOILS

Soil treatment	Distance from original place of application of inoculum, in.	Average length of wheat plants, cm.	Average degree of infection of wheat plants, %	Results of re-isolation trials of *F. graminearum*
Soil left bare. Soil + cornmeal with no organism placed at one end of box. Included to check against natural occurrence of *F. graminearum* in the soil.	0	32.0	1.1	−
	2	26.2	3.6	−
	4	28.3	1.1	−
	6	25.5	1.0	−
	8	24.2	4.8	−
	10	24.7	4.4	−
	12	26.6	2.3	−
	14	26.9	3.4	−
	16	27.2	1.1	−
	18	26.6	2.5	−
Soil left bare. *F. graminearum* placed at one end of box.	0	27.5	11.9	+
	2	28.5	3.8	−
	4	27.9	12.6	−
	6	27.6	2.4	−
	8	27.1	1.3	+
	10	27.4	5.0	−
	12	22.6	1.0	−
	14	27.2	6.3	+
	16	22.6	3.2	+
	18	27.1	1.1	−
Soil seeded to wheat. *F. graminearum* placed at one end of box.	0	27.9	24.8	+
	2	27.6	16.9	+
	4	25.1	24.1	−
	6	23.0	17.4	−
	8	22.2	19.0	−
	10	20.8	22.9	−
	12	19.0	19.4	−
	14	22.3	12.1	+
	16	22.2	16.6	−
	18	21.3	16.7	−
Soil seeded to *A. tenerum*. *F. graminearum* placed at one end of box.	0	26.7	27.9	+
	2	26.5	21.2	+
	4	25.8	21.4	+
	6	23.4	26.1	−
	8	22.6	16.7	−
	10	24.1	12.9	+
	12	24.1	11.1	+
	14	24.0	10.9	−
	16	22.5	17.2	−
	18	22.9	18.5	−
Soil seeded to *A. repens*. *F. graminearum* placed at one end of box.	0	26.6	26.9	+
	2	27.2	27.5	+
	4	25.2	23.2	−
	6	24.9	30.8	+
	8	23.1	32.4	−
	10	24.4	27.4	−
	12	24.0	32.1	−
	14	23.5	23.9	−
	16	24.1	31.0	−
	18	22.9	17.2	+

Experimental Results

The average length and the average degree of infection in per cent of each row of wheat plants, together with the results of the isolations, are given in detail in Tables V and VI. They are also summarized in Tables II and III, in which the plant lengths and degrees of infection given are the averages for the plants of all three replicates grouped together. The distances of spread of *O. graminis* in each box under the various treatments are shown in Table IV.

TABLE IV

DISTANCES WHICH *O. graminis* SPREAD IN TWO MONTHS IN BARE SOIL AND IN SOIL SEEDED TO *A. tenerum*, *A. repens*, AND WHEAT

Soil treatment	Distance which *O. graminis* spread in the soil (to nearest 2 in.)		
	Series 1, in.	Series 2, in.	Series 3, in.
Bare soil	0	2	0
Wheat	6	8	8
A. tenerum	4	12	8
A. repens	4	4	4

The results with *O. graminis* are striking and significant. Not only are there appreciable differences in the distances the organism spread under different conditions, but also there is, in most cases, quite close agreement between the replicates of each series. Table V shows that the fungus spread in only one of the three boxes of unplanted soil, and there it only spread about two inches and caused a small amount of infection of wheat. In boxes growing wheat it spread about six inches in one box and eight inches in the other two; under *A. tenerum* it spread from four to twelve inches; and in all boxes of *A. repens* it spread four inches. The organism was in no instance isolated from wheat plants from the uninfested control boxes.

Results with *F. graminearum* were irregular and less conclusive. While considerable care was taken in watering the boxes, they were not sloped and some washing of soil no doubt occurred. The effects of this were accentuated by the presence of spores in the inoculum. That these spores were carried for considerable distances is suggested by the fact that single infected plants were found isolated from other infected plants by as much as fourteen inches in one instance. The *F. graminearum* causing this infection evidently came from the original inoculum, since in no instance was the organism isolated from uninfested soil. It is unfortunate that the wheat plants for isolation were allowed to grow for so long a period before they were removed from the soil. Especially in the last two series harvested there was so heavy an infection with *Helminthosporium sativum* (indicated by H in the isolation column) occurring naturally in the soil that the degrees of infection recorded in the columns of Table VI do not give a true picture of the action of *F. graminearum*. The chief value of these columns is in demonstrating the increase in damage caused by *H. sativum* in naturally infested soil following wheat, *A. tenerum*, and *A. repens*. It can only be said that there is no definite evidence that the spread of *F. graminearum* in unsterilized soil is affected by the presence of susceptible plants in the soil.

TABLE V

RESULTS OF EXPERIMENTS ON THE SPREAD OF *O. graminis* IN BOXES OF SOIL

Soil treatment	Distance from inoculum, in.	First replicate			Second replicate			Third replicate		
		Length, cm.	Degree of infection, %	Isolations	Length, cm.	Degree of infection, %	Isolations	Length, cm.	Degree of infection, %	Isolations
Soil left bare. Soil + cornmeal, with no organism, placed at one end of box. Included to check against natural occurrence of *O. graminis* in soil.	0	26.9	0	−	26.6	0	−	23.7	0	−
	2	25.2	0	−	28.4	0	−	25.9	0	−
	4	22.1	0	−	27.1	0	−	21.9	0	−
	6	25.4	0	−	21.4	0	−	21.1	0	−
	8	25.9	0	−	23.4	0	−	21.1	0	−
	10	29.0	0	−	22.4	0	−	21.8	0	−
	12	23.5	0	−	20.6	0	−	21.6	0	−
	14	23.3	0	−	23.8	0	−	21.6	0	−
Soil left bare. *O. graminis* placed at one end of box.	0	26.1	14.5	+	20.4	15.0	+	25.9	13.3	+
	2	24.4	0	−	24.0	4.0	+	23.6	0	−
	4	25.6	0	−	24.1	0	−	21.9	0	−
	6	25.3	0	−	22.4	0	−	23.2	0	−
	8	26.0	0	−	22.8	0	−	27.4	0	−
	10	20.4	0	−	24.1	0	−	24.5	0	−
	12	22.5	0	−	21.2	0	−	22.6	0	−
	14	25.8	0	−	22.5	0	−	22.6	0	−
Soil seeded to wheat. *O. graminis* placed at one end of box.	0	29.2	26.7	+	26.7	10.0	+	30.9	4.0	+
	2	25.4	32.0	+	22.5	42.5	+	23.9	16.0	+
	4	18.2	38.2	+	21.4	24.0	+	20.5	26.2	+
	6	20.7	16.9	+	20.1	10.9	+	17.0	18.2	+
	8	22.0	11.4	−	21.6	8.3	+	22.4	3.1	+
	10	22.3	1.7	−	20.6	0	−	20.5	0	−
	12	20.7	0	−	19.9	0	−	22.9	0	−
	14	22.9	0	−	22.8	0	−	18.9	0	−
Soil seeded to *A. tenerum*. *O. graminis* placed at one end of box.	0	27.2	5.4	+	24.9	14.7	+	25.0	7.3	+
	2	22.6	32.3	+	19.5	27.7	+	22.9	13.3	+
	4	22.2	10.0	+	20.6	13.3	+	20.9	9.1	+
	6	20.0	0	−	17.0	25.5	+	19.4	1.8	+
	8	19.3	0	−	17.4	17.8	+	21.2	2.8	+
	10	16.9	0	−	17.1	1.8	+	21.0	0	−
	12	18.4	0	−	20.6	1.5	+	17.1	0	−
	14	19.4	0	−	18.9	0	−	18.2	0	−
Soil seeded to *A. repens*. *O. graminis* placed at one end of box.	0	24.8	15.3	+	27.7	6.2	+	26.4	4.4	+
	2	29.6	26.7	+	26.5	22.5	+	23.5	2.2	+
	4	21.3	18.7	+	20.8	11.4	+	21.5	6.7	+
	6	21.6	0	−	20.1	3.3	−	20.5	0	−
	8	19.7	0	−	20.4	0	−	21.1	0	−
	10	21.7	0	−	20.6	0	−	20.5	0	−
	12	20.8	0	−	19.4	0	−	21.5	0	−
	14	16.5	0	−	18.5	0	−	19.1	0	−

Discussion

There is ample evidence that susceptible species tend to aid *O. graminis* to survive in the soil and to bring about an increase of the amount of inoculum of this organism for succeeding wheat crops. In the case of *H. sativum* the effects are somewhat less marked, but there is sufficient evidence to conclude that *Bromus inermis* as well as some species of *Agropyron* increase the amount

TABLE VI

RESULTS OF EXPERIMENTS ON THE SPREAD OF *F. graminearum* IN BOXES OF SOIL

Soil treatment	Distance from inoculum, in.	First replicate			Second replicate			Third replicate		
		Length, cm.	Degree of infection, %	Isolations	Length, cm.	Degree of infection, %	Isolations	Length, cm.	Degree of infection, %	Isolations
Soil left bare. Soil + cornmeal, with no organism placed at one end of the box. Included to check against natural occurrence of *F. graminearum* in the soil.	0	27.4	0	− −	33.0	0	− −	30.8	2.7	− −
	2	23.9	0	− −	28.4	8.0	− H	28.0	5.5	− H
	4	22.9	0	− −	30.7	0	− −	30.5	3.6	− −
	6	18.5	0	− −	27.7	0	− −	29.9	2.9	− −
	8	23.0	0	− −	23.3	8.3	− H	27.5	5.7	− H
	10	24.6	0	− −	24.5	6.0	− H	25.0	8.9	− −
	12	22.7	1.4	− −	29.1	1.7	− −	29.3	4.4	− H
	14	24.4	0	− −	29.4	8.0	− H	26.9	2.2	− −
	16	23.6	0	− −	29.1	1.5	− −	30.1	2.5	− −
	18	24.4	0	− −	29.3	0	− −	27.0	10.0	− H
Soil left bare. *F. graminearum* placed at one end of the box.	0	24.6	5.0	+ −	26.3	20.0	+ H	34.1	13.3	+ −
	2	24.1	0	− −	29.9	9.3	− H	33.1	1.8	− −
	4	25.6	2.4	− H	27.3	0	− −	31.8	1.5	− −
	6	27.0	1.3	− −	26.7	5.7	− −	29.2	0	− −
	8	26.3	1.2	+ −	28.3	3.1	− H	27.1	0	− −
	10	26.0	8.3	− −	27.5	4.0	− H	29.0	2.2	− H
	12	24.2	0	− −	28.6	3.3	− −	27.0	0	− −
	14	25.8	3.6	+ −	27.3	8.6	− H	28.3	6.2	− H
	16	22.1	4.7	+ −	28.2	5.5	− H	27.4	0	− −
	18	24.8	0	− −	28.6	3.3	− H	27.9	0	− −
Soil seeded to wheat. *F. graminearum* placed at one end of the box.	0	23.5	29.1	+ −	27.9	37.5	+ H	32.7	10.0	+ H
	2	22.3	8.3	− H	30.3	29.3	− H	29.4	10.0	+ H
	4	21.4	14.0	− H	25.8	40.0	− H	27.9	8.9	− H
	6	19.3	9.1	− H	22.9	28.0	− H	26.1	12.3	− H
	8	21.3	10.0	− H	21.4	30.7	− H	23.8	14.3	− H
	10	20.7	8.6	− H	19.4	50.0	− H	22.5	9.2	− H
	12	18.8	7.5	− H	18.8	43.6	− H	19.4	12.0	− H
	14	17.6	11.7	− H	23.0	16.5	+ H	25.5	7.1	− H
	16	21.3	3.3	− −	20.6	35.7	− H	24.3	9.3	− H
	18	20.3	9.2	− −	20.2	36.4	− H	23.4	6.7	− H
Soil seeded to *A. tenerum*. *F. graminearum* placed at one end of the box.	0	22.3	16.0	+ H	24.5	49.1	+ H	32.3	18.3	− H
	2	24.4	20.0	+ H	24.9	35.6	+ H	29.5	12.3	− H
	4	23.7	18.7	+ H	24.4	40.0	+ H	28.4	12.9	− H
	6	20.2	25.0	− H	23.8	38.3	− H	26.3	15.0	− H
	8	23.2	10.0	− H	22.6	23.1	− H	22.0	17.3	− H
	10	22.2	0	− −	22.7	18.3	+ H	25.7	14.4	− H
	12	23.7	5.7	− H	24.2	11.4	+ H	24.5	16.0	− H
	14	22.8	7.3	− H	24.0	14.5	− H	25.1	10.9	− H
	16	21.9	1.8	− −	23.8	28.3	− H	21.7	20.0	− H
	18	21.7	12.0	− H	23.6	27.7	− H	23.0	12.0	− H
Soil seeded to *A. repens*. *F. graminearum* placed at one end of the box.	0	21.8	38.0	+ H	31.3	13.3	+ H	27.2	28.0	+ H
	2	22.5	15.6	+ H	25.0	36.9	− H	34.4	26.0	− H
	4	21.6	15.0	− H	27.7	21.4	− H	27.7	35.6	− H
	6	23.3	14.5	− H	25.1	48.3	− H	26.0	28.6	+ H
	8	17.0	40.0	− −	24.6	38.3	− H	25.3	22.9	− H
	10	22.5	21.4	− H	24.3	41.5	− H	27.7	15.0	− H
	12	19.9	32.0	− H	24.9	43.1	− H	27.0	18.0	− H
	14	22.9	11.1	− −	22.5	36.0	− −	25.1	18.3	− H
	16	21.6	4.0	− H	24.8	36.7	− H	25.9	13.3	− H
	18	21.8	6.2	+ H	20.8	31.1	− H	25.4	18.5	− H

of inoculum of this organism in the soil, and since the fungus seems to be of widespread distribution, it seems probable that such grasses will play a part in the foot rotting of wheat by this organism also. There is a fair amount of evidence that *Agropyron* species do not appreciably increase the amount of inoculum of *F. graminearum*. *Bromus inermis*, on the other hand, may be of importance. No evidence has yet indicated that resistant or immune species can be expected actually to have a detrimental effect on the foot-rotting fungi.

Survey work conducted in the province of Alberta has indicated that the damage caused by take-all is probably greater than that caused by other foot-rotting organisms, and for this reason special emphasis should be laid on the take-all problem. It is evident from the results that in order to control take-all, susceptible species of grasses should be avoided or used sparingly in rotations. Experimental results and field observations show that old stands of slender wheat grass have a marked effect upon the accumulation of *O. graminis*, and for this reason cultivation of this crop should not be encouraged where the take-all disease presents a very serious problem in wheat culture. It has been seen that *A. repens* occurs often even in fallow fields where other weeds have been kept under control. Rotations which do not involve eradication of this grass, and summerfallow methods which do not destroy it, cannot be expected to solve the problem, and may even intensify it. From knowledge so far accumulated it would seem that awnless brome grass may perhaps offend less in this respect than slender wheat grass, but it certainly is not entirely free from blame.

The experiments shed light on the manner in which *O. graminis* spreads in the soil. This organism spreads little, if at all, in bare soil, but in soil supporting growth of susceptible grasses it spread a considerable distance. It is true that the greatest spread which occurred was only twelve inches in six weeks, but when it is considered that during this short period it had to establish itself upon healthy young plants in the soil and pass from plant to plant presumably by means of the fine network of roots, that no attempt was made to provide ideal conditions for the growth of the organism in the soil, that previous attempts to demonstrate the spread of the organism had resulted in failure, and that in the unplanted soil the degree of infection of wheat seedlings grown actually in the inoculum six weeks after it was applied was much lighter than that of plants two and four inches distant in planted soil, there is abundant evidence that susceptible species aid greatly in the spread of the organism, if, indeed, they are not essential.

It is of interest to compare this lateral growth of *O. graminis* with that of *Phymatotrichum omnivorum* (15), which has been found to spread radially in all directions from infected cotton plants at the astounding rate of 4½ metres in 50 days. Recently it has been demonstrated at the Rubber Research Institute of Malaya (19) that rhizomes of the rubber root-rot fungi *Fomes lignosus*, *Ganoderma pseudoferreum* and *Fomes noxius* do not grow directly through the soil, but require as a vehicle a chain of solid surfaces, preferably the surfaces of roots. There appears to be much in common among these various parasitic fungi which have such widely different hosts.

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REACTION OF BARLEY VARIETIES TO INFECTION WITH COVERED SMUT (*USTILAGO HORDEI* PERS. K & S)[1]

By O. S. Aamodt[2] and W. H. Johnston[3]

Abstract

Results are presented of tests conducted at the University of Alberta, Edmonton, during the years 1931-34, to determine the relative resistance of barley varieties to the covered smut disease caused by *Ustilago hordei*.

Extensive field trials, including 138 varieties, carried out in 1931, with hulled seed, gave inconclusive results owing to low infection percentages. Junior and Eureka, two naturally hulless varieties, evidenced high susceptibility with 66 and 42% infection respectively. Field tests of a number of standard varieties in 1932, in which the seed was dehulled with sulphuric acid, resulted in an increase in the percentage of smutted plants. Unfortunately, the acid treatment of the kernels caused a general impairment in germination which lessened somewhat the significance of the results obtained. In 1934, the comparative infections and stands of varieties grown from hulled, hand-dehulled, scarified and acid-dehulled seed were determined. The data were treated statistically by the analysis of variance method. Significant variations due to varieties, treatments and interaction of varieties and treatments were obtained with regard to both percentage infection and percentage stand. All three of the dehulling measures increased smut infection significantly. Highest infection percentages resulted from hand-dehulled seed, followed by acid-dehulled and scarified seed in the order mentioned.

The least reduction in stand resulted from hulled seed and the greatest from acid-dehulled seed. Scarified and hand-dehulled seed gave stands intermediate in numbers. Distinct varietal differences existed in thickness of hull or in the resistance of the hull to acid treatment. The average percentage stands of the different varieties tended to be directly proportional, and the average percentage smut infection inversely proportional to the amount of hull remaining on the kernels following acid treatment. Varieties grown from acid-dehulled and scarified seed were found to be delayed in heading 1½ and 2½ days respectively as compared with varieties grown from hulled or hand-dehulled seed. There was a tendency for the later varieties to be more susceptible to covered smut than the earlier ones.

The varieties used in these investigations differed greatly in their reaction to covered smut. A fair degree of correlation was found to exist between the varietal infection percentages induced in 1932 by acid-dehulled seed and those induced by either hand-dehulled or acid-dehulled seed in 1934.

Two distinct physiologic forms of *U. hordei* were found in collections gathered from six points in central Alberta. These are readily distinguished by their reaction on the varieties Eureka and Canadian Thorpe or Hannchen.

From the experimental data it was concluded that the following varieties showed resistance to covered smut:

Six-rowed, hulled types—O.A.C. No. 21, Atlas, Sacramento, Glabron, Velvet, Leiorrhynchum, Wisconsin Barbless No. 38, Shaw, Sol and Success.

Two-rowed, hulled types—Spartan, Golden Pheasant and Horn.

Hulless types—Himalayan, New Era, Russian, Mongolian and Burbank.

The following varieties showed susceptibility to one or more of the smut collections used:

Six-rowed, hulled types—Bearer, Lapland, Star, Manchurian, Peatland, Trebi, Silver King, Vaughn, Comfort, Regal, Newal and Colsess.

Two-rowed, hulled types—Binder, Canadian Thorpe, Duckbill, Gold, Hannchen, Swanneck and Charlottetown.

Hulless types—Junior, Eureka, Improved White Hulless and Trifurcatum.

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Introduction

For some time the Department of Field Crops, University of Alberta, has been testing the reaction of barley varieties to infection with the more common diseases of the crop occurring in western Canada, in order that improvement of already existing strains may be undertaken. This paper summarizes the results obtained in regard to varietal reaction to infection with covered smut, *Ustilago hordei*.

The losses from the barley smuts in Canada are much greater than is generally appreciated. During the period 1920-23 Canadian farmers lost over one million dollars annually from these diseases (8). Covered smut (*U. hordei*) is a widespread disease of the barley crop in western Canada, but appears to be particularly destructive in Alberta (3, 4, 13). In 1931, 53% of the fields examined in Alberta were smutty; the average damage being 2.3%. The two highest infections reported were 25 and 70% (3). Covered smut was present in 33% of the fields examined in 1932 and 1933. The average damage in infested fields was 2.9% in 1932 and 1.9% in 1933. Infections of 15 and 30% were recorded in 1932, while the highest infection reported for 1933 was 10% (3).

Varietal Reaction

Literature Review

The literature concerning varietal resistance to the covered smut disease is comparatively fragmentary, and the results reported largely inconclusive. Briggs (1) refers to extensive tests carried out at the California Agricultural Experiment Station in which practically no infection resulted, although the seed had been heavily inoculated with smut. Tisdale (15) in Virginia was unable to determine the resistance of varieties to covered smut because of a lack of satisfactory infection. Conners (2) also reports inconclusive results after testing over a hundred varieties and strains. Rodenhiser (14) refers to investigations carried out at University Farm, St. Paul, Minnesota, during the years 1924 to 1927, in which about 135 varieties and selections of barley were treated for their reaction to covered smut. Although the varieties were heavily inoculated, so little smut developed in most of them that the results could not be considered significant. Observations made at about the same time, on the reaction of varieties in other plots at the Minnesota Agricultural Experiment Station, and at a number of its Branch Stations, yielded more definite information regarding varietal resistance to the covered smut disease. Lion proved immune, and White Spring Hulless was highly susceptible. The varieties Odessa, Trebi, Glabron and Svanhals showed moderate susceptibility, while Manchuria and Svansota were classed as resistant. Hanna and Popp (9), working at Winnipeg, Manitoba, have contributed some definite information regarding the reaction to *U. hordei* of four varieties commonly grown in western Canada. They report that the varieties Hannchen, Canadian Thorpe and Trebi evidenced susceptibility to the collection of smut used, while O.A.C. No. 21 was free from smutted heads.

As far as could be determined from the literature, the above-mentioned investigators used inoculum with a high degree of viability. It is therefore evident that other factors, about which little seems to be known, are important contributing factors influencing successful infection of barley. Tisdale (17) showed that greatly increased infection may be secured by removing the hull prior to inoculation. This finding was corroborated by Faris (6) and Briggs (1). Faris (5) demonstrated that high infection could be obtained on the Hannchen variety at soil moistures and temperatures generally existing at the time of seeding. He states, furthermore, that the biologic form of the smut used may have more bearing on successful infection than any soil conditions. Taylor and Zehner (16) found that in winter barley greater covered smut infection resulted from deep than from shallow seeding. Rump, cited by Leukel (12) found that an alkali soil stimulated covered smut development, that acid soil was injurious to it and that a soil moisture content of 20% was the optimum for development of covered smut. Schaffnit, also cited by Leukel (12), reported that a soil rich in organic matter and carbonic acid favored covered smut infection.

VARIETAL TESTS, 1931

Methods

Extensive tests to determine the resistance of barley varieties to covered smut were made in 1931. No attempt was made to remove the hull in the case of hulled varieties.* Approximately 75 inoculated seeds of each variety were sown in 10-foot rows. The inoculum consisted of a composite sample of chlamydospores gathered in 1930 from the rod-row varietal plots. The smutted heads were run through an ordinary meat grinder, and the powdered spores dusted on the seed just previous to sowing. All seeding was done in the latter half of May after the soil had reached a temperature of approximately 15-20° C. The first test included 138 varieties and strains from the barley classification nursery grown at the University of Alberta.

Experimental Results

The results of this experiment are in accord with those reported by earlier workers in that, owing to low infection, it is difficult to draw definite conclusions regarding varietal reaction. The infection percentages for 32 of the 138 varieties in this test are given in Table I as "Experiment A". The only variety showing extreme susceptibility was Junior, with 66% infection. Other varieties exhibiting more than 10% infection are listed below, with their respective infection percentages:

Six-rowed hulless types—Trifurcatum, 21%; Eureka, 18%; Improved White Hulless, 14%.

Six-rowed hulled types—Silver King, 15%; Garton's No. 68, 11%; Sans Barb Early, 11%.

Two-rowed hulled types—Alpha, 13%; Danish Island, 12%.

* *Throughout this paper, the current practice is being followed of referring to "hulled" seed or varieties as normal grain with the hull intact; "hulless" as normal grain without the hull attached to the caryopsis, and "dehulled" as seed of hulled varieties from which the hulls have been removed artificially.*

Trebi and O.A.C. No. 21, two commonly grown six-rowed barleys, showed only 2% infection. The two-rowed barleys, Canadian Thorpe and Hannchen, exhibited 5 and 9% covered smut respectively. Of the smooth-awned varieties tested, Glabron, Velvet and Regal failed to show any smutted plants, while Comfort had 5%.

TABLE I

REACTION OF SOME COMMON BARLEY VARIETIES TO INFECTION WITH COVERED SMUT (*U. hordei*) AS DETERMINED BY FIELD TESTS CONDUCTED IN 1931 AT THE UNIVERSITY OF ALBERTA, EDMONTON

Variety and group	Number*	Percentage of plants infected	
		Experiment A	Experiment B
Six-rowed, hulled, rough-awned types			
Atlas	C.A.N. 702	–	0
Bark's	C.A.N. 703	0	1
California Mariout	C.A.N. 1083	0	0
Garton's No. 68	C.A.N. 1033	11	–
Manchurian	C.A.N. 726	5	7
Minsturdi	C.A.N. 732	2	0
O.A.C. No. 21	C.A.N. 734	2	0
Peatland	C.A.N. 722	2	15
Sacramento	C.A.N. 744	–	0
Silver King	C.A.N. 1048	15	–
Star	C.A.N. 748	0	5
Trebi	C.A.N. 753	2	4
Vaughn	C.A.N. 759	–	0
Vaughn	C.A.N. 1090	0	0
Six-rowed, hulled, smooth-awned types			
Comfort	C.A.N. 712	5	0
Glabron	C.A.N. 718	0	0
Regal	C.A.N. 742	0	0
Sans Barb Early	N.S.N. I-31-7	11	–
Newal	C.A.N. 1089	–	2
Velvet	C.A.N. 755	0	0
Six-rowed, hulled, hooded types			
Colsess	C.A.N. 772	4	2
Sol	C.A.N. 782	–	6
Six-rowed, hulless types			
Eureka	C.A.N. 773	18	42
Hulless	N.S.N. I-31-6	–	0
Improved White Hulless	C.A.N. 1056	14	–
Junior	C.A.N. 786	66	–
New Era	C.A.N. 721	–	3
Trifurcatum	C.A.N. 891	21	–
Two-rowed types			
Alpha	C.A.N. 801	13	–
Alberta Beardless (hooded)	C.A.N. 874	8	4
Canadian Thorpe	C.A.N. 816	5	6
Charlottetown No. 80	C.A.N. 817	4	3
Danish Island	C.A.N. 1002	12	–
Duckbill	C.A.N. 826	2	12
Gold	C.A.N. 829	2	6
Hannchen	C.A.N. 837	9	7
Horn	C.A.N. 1078	0	0
Plumage Archer	C.A.N. 1004	0	4
Spartan (smooth awn)	C.A.N. 860	0	3

**C.A.N. = Canadian Accession Number; N.S.N. = University of Alberta Nursery Stock Number.*

A second experiment, "B", was conducted in which the reaction to covered smut infection was determined for 31 of the standard varieties. These varieties, together with their infection percentages, are given in Table I as "Experiment B".

Eureka, a hooded, hulless variety, showed considerable susceptibility with 42% infection. Peatland, a six-rowed, hulled, bearded type, and Duckbill, a two-rowed, hulled, bearded type, were the only other varieties displaying susceptibility. No smutted plants were found in O.A.C. No. 21, Atlas, Minsturdi, Sacramento, Vaughn, Glabron, Velvet and Regal. Canadian Thorpe and Hannchen showed 6 and 7% infection respectively.

The relatively high infection resulting on the naturally hulless varieties indicated that both inoculum and environmental conditions were favorable for successful infection. Furthermore, what is more important, it suggests that the presence of the hull was possibly a deterrent to successful infection in the case of hulled varieties. To investigate this possibility, a second varietal test was conducted in 1932, in which the seed was dehulled.

VARIETAL TESTS, 1932

Methods

The kernels of the varieties used in this test were dehulled by means of sulphuric acid. The feasibility of using this chemical to dehull barley kernels in order to induce infection with covered smut, was suggested by Briggs (1). The technique used in dehulling the kernels in these experiments was one developed by the junior author while studying the inheritance of reaction to covered smut infection in certain barley crosses (11).

The kernel lots were immersed in concentrated sulphuric acid for a period of two minutes. Following this they were rinsed with cold water and returned to the acid for 10 seconds. The action of the acid on the wet seed coats caused a rapid and more or less complete removal of the hull from the greater part of the kernel. The dehulled kernels were then washed thoroughly with cold water for two or three minutes. In an effort to remove all traces of acid, the kernel lots were placed in a concentrated solution of $NaHCO_3$ (baking soda) for several minutes. After another rinse with cold water they were placed on blotting paper to dry.

Two series of approximately 50 kernels of each variety were dehulled by acid. One series was inoculated with a composite of covered smut chlamydospores collected from the varietal plots at Edmonton in 1931. The other was dusted with chlamydospores from a collection made in a commercial field of smooth-awned barley at Winterburn, Alberta.

The inoculated seed of both replicates was sown in 10-foot rows, with soil temperatures of approximately 16° C. Unfortunately, the viability of the acid-treated seed was generally low; this being reflected in reduced stands. The kernels of some of the two-rowed barleys appeared especially susceptible to acid injury. For this reason it was necessary to include in the data the total number of plants, in addition to the percentage infected.

Experimental Results

The data in Table II summarize the reaction of 32 varieties exposed to both the Edmonton and Winterburn smut collections. To facilitate examination and discussion, the varieties have been grouped into several commonly

TABLE II

REACTION OF SOME COMMON BARLEY VARIETIES TO TWO COLLECTIONS OF COVERED SMUT, WHEN GROWN FROM SEED DEHULLED WITH SULPHURIC ACID, AND SOWN IN THE FIELD AT THE UNIVERSITY OF ALBERTA, EDMONTON, 1932

Variety and group	Number*	Smut from			
		Edmonton		Winterburn	
		Total number of plants	Percentage of plants infected	Total number of plants	Percentage of plants infected
Six-rowed, hulled, rough-awned types					
Bearer	C.A.N. 704	23	9	10	0
Lapland	N.S.N. I-32-2	32	19	29	31
Manchurian	C.A.N. 726	29	55	14	14
Minsturdi	C.A.N. 732	26	8	18	0
O.A.C. No. 21	C.A.N. 734	42	2	27	11
Peatland	C.A.N. 722	17	24	25	16
Sacramento	C.A.N. 744	10	0	14	0
Star	C.A.N. 748	10	20	12	25
Trebi	C.A.N. 753	36	25	27	11
Vaughn	C.A.N. 759	29	21	21	5
Six-rowed, hulled, smooth-awned types					
Comfort	C.A.N. 712	41	10	35	0
Glabron	C.A.N. 718	38	0	42	0
Newal	C.A.N. 1089	25	28	24	25
Regal	C.A.N. 742	42	17	27	7
Velvet	C.A.N. 755	36	0	28	0
Wisconsin Barbless No. 38	C.A.N. 758	23	9	22	0
Six-rowed, hulled, hooded types					
Colsess	C.A.N. 772	35	9	25	4
Sol	C.A.N. 782	16	13	10	0
Success	N.S.N. I-32-4	26	0	13	0
Six-rowed, hulless types					
Eureka	C.A.N. 773	65	15	50	0
Himalayan	N.S.N. I-32-3	54	2	32	0
New Era	C.A.N. 721	58	5	50	0
Two-rowed types					
Alberta Beardless (hooded)	C.A.N. 874	12	8	14	0
Binder	N.S.N. I-32-8	32	0	25	0
Canadian Thorpe	C.A.N. 816	14	7	13	23
Charlottetown No. 80	C.A.N. 817	20	45	24	29
Duckbill	C.A.N. 826	10	30	12	33
Gold	C.A.N. 829	9	11	17	41
Golden Pheasant	N.S.N. I-32-9	28	0	19	0
Hannchen	C.A.N. 837	14	36	7	0
Horn	C.A.N. 1078	48	2	21	10
Spartan (smooth-awned)	C.A.N. 860	15	13	14	29

* *C.A.N. = Canadian Accession Number; N.S.N. = University of Alberta Nursery Stock Number.*

recognized classes. In general, the infection percentages of varieties with stands of less than 20 plants have been considered as too unreliable to merit consideration. As has been indicated previously, the individual plant was used as the basis for the calculation of infection percentages.

A general examination of the data in Table II shows that the infection resulting from the Edmonton collection of smut is generally more severe than that resulting from the Winterburn collection. The average percentage infection of the 32 variteies when inoculated with the Edmonton smut collection was 13.8; with the Winterburn collection it was 9.5.

Reaction of six-rowed, hulled, rough-awned types. Manchurian, with 55% infection, was the most susceptible to the Edmonton sample of smut. Trebi, Vaughn and Lapland displayed moderate susceptibility to the smut, with infection percentages ranging from 19 to 25. Lapland showed considerable susceptibility to the Winterburn collection, while Peatland, O.A.C. No. 21 and Trebi were moderately so. Bearer appeared highly resistant to both collections. No smutted plants were found in either replicate of Sacramento, but the reduced population in these instances makes any definite statement regarding resistance unreliable.

Reaction of six-rowed, hulled, smooth-awned types. High resistance to both collections of smut was displayed by Glabron and Velvet, while Newal and Regal proved susceptible. Comfort and Wisconsin Barbless No. 38 showed moderate resistance to the Edmonton collection of smut, and produced no smutted plants when inoculated with the Winterburn collection.

Reaction of six-rowed, hulled, hooded types. Colsess and Sol were moderately resistant, and Success was completely resistant to the covered-smut collections used.

Reaction of six-rowed, hulless types. The varieties New Era and Himalayan displayed high resistance to both collections of smut used. Eureka proved to be moderately susceptible to the Edmonton collection and highly resistant to the one from Winterburn.

Reaction of two-rowed types. No smut appeared on either replicate of Binder and Golden Pheasant. Horn appeared to be highly resistant to the Edmonton collection but only moderately so to the one from Winterburn. Charlottetown, on the other hand, showed high susceptibility to both, with infections of 45 and 29%. The varieties Hannchen, Duckbill, Gold, Canadian Thorpe and Spartan appeared to be susceptible to one or both of the collections used; but again inadequate populations render a definite statement regarding resistance impossible.

No smut tests were made on hulled seed in 1932, consequently it is difficult to state how much the dehulling increased the susceptibility of the various varieties to infection. The results do indicate, however, that considerably higher smut percentages are obtained when the hull is partially removed by acid. This method of dehulling resulted in reduced stands, which lessened

somewhat the significance of the data obtained. In general the results of these experiments are in accord with those of earlier workers (1, 15), in that results obtained from extensive barley smut tests, using hulled seed, are not entirely satisfactory.

In order to obtain further information on the best method to use in dehulling seed, and its effectiveness in increasing smut infections of various barley varieties, another experiment was conducted in 1934.

Varietal Tests, 1934

Methods

In 1934, the reaction to covered smut was determined for 25 hulled varieties of barley. Three separate methods of dehulling the kernels were employed:

i. Hand removal of hull over the region of the embryo with a scalpel.

ii. Scarification with sandpaper. This was accomplished by rubbing the seed between two sheets of sandpaper of medium coarseness. This method of dehulling removed the hull quite effectively from the dorsal and ventral sides of the kernels, but did not expose the embryos.

iii. Digestion of hull with concentrated sulphuric acid. The details of the method employed in this instance were similar to those used in dehulling the kernels in the 1932 tests.

Hulled samples of each variety were also prepared to serve as checks, making in all four series of kernels.

Duplicate lots of 75 kernels were used in each treatment and the plots were systematically arranged. The inoculum consisted of a composite sample of smutted spikes obtained from the 1933 varietal plots. Again the plant, rather than the spike, was used as the basis of determining infection percentages. All plants showing any degree of infection were considered susceptible.

Experimental Results

Before considering the specific varietal reaction to covered smut indicated in this trial, it is proposed to discuss the relative merits of the four methods of treating the seed prior to inoculation as outlined in the previous section. Special attention will be given their relative values in inducing smut infection, their effect on emergence or final stand, and their effect on plant development. A considerable number of correlation coefficients were calculated in order to show the similarity of the different treatments on the varieties, and to indicate the reliability of the results. For the sake of convenience and clarity each pair of variables correlated has been assigned a number and is listed in numerical order in Table VI. Reference will be made by number to any correlation under discussion.

Effect of dehulling on covered smut infection. The average infection percentages of the different varieties for each of the four treatments is given in Table III. It is evident that much lower infections resulted from hulled

TABLE III

AVERAGE PERCENTAGE SMUT INFECTION WITH *U. hordei* AND AVERAGE PERCENTAGE STANDS OF 25 VARIETIES GROWN FROM HULLED, HAND-DEHULLED, SCARIFIED AND ACID-DEHULLED SEED IN 1934

Variety	Number*	Average percentage stand and infection with *U. hordei*								Mean percentage stand (8 plots)	Mean percentage infection (8 plots)
		Hulled		Hand-dehulled		Scarified		Acid-dehulled			
		Stand	Infection	Stand	Infection	Stand	Infection	Stand	Infection		
Six-rowed, hulled, rough-awned types											
Atlas	C.A.N. 702	87	0	80	1	85	0	75	1	81.6	0.5
Bearer	C.A.N. 704	95	2	75	26	68	3	49	28	74.5	14.4
Lapland	N.S.N. I-32-2	71	12	39	45	35	31	17	32	40.7	29.6
Manchurian	C.A.N. 726	93	8	51	53	40	28	15	46	49.4	33.5
O.A.C. No. 21	C.A.N. 734	89	1	80	1	56	3	41	4	66.3	2.1
Peatland	C.A.N. 722	84	1	76	22	48	18	23	16	57.4	14.1
Trebi	C.A.N. 753	83	1	76	35	77	16	63	21	74.3	18.1
Vaughn	C.A.N. 759	81	5	61	4	60	0	57	8	64.9	4.3
Six-rowed, hulled, smooth-awned types											
Comfort	C.A.N. 712	81	3	61	21	43	2	61	6	61.3	7.6
Glabron	C.A.N. 718	96	0	76	9	71	2	67	1	77.4	3.0
Newal	C.A.N. 1089	91	4	36	31	57	12	56	7	60.0	13.3
Leiorrhynchum	N.S.N. I-32-11	89	0	40	3	77	4	68	4	68.1	2.8
Regal	C.A.N. 742	81	0	69	9	63	9	57	8	67.4	6.4
Velvet	C.A.N. 755	91	1	71	6	55	0	55	3	67.6	2.3
Wisconsin Barbless No. 38	C.A.N. 758	77	0	69	1	65	0	60	0	67.9	0.3
Six-rowed, hulled, hooded types											
Colsess	C.A.N. 772	81	5	68	39	67	21	33	33	61.8	24.4
Shaw	C.A.N. 1047	84	1	51	11	53	9	27	14	53.5	8.5
Sol	C.A.N. 782	77	3	52	9	39	12	39	4	50.1	6.8
Two-rowed, hulled types											
Binder	N.S.N. I-32-8	91	1	76	46	72	15	47	20	71.0	20.1
Canadian Thorpe	C.A.N. 816	88	1	79	33	53	24	32	40	62.5	24.5
Duckbill	C.A.N. 826	92	11	77	21	68	24	57	4	73.4	14.6
Gold	C.A.N. 829	84	2	79	50	59	10	33	49	63.4	27.5
Hannchen	C.A.N. 837	87	6	87	60	80	19	41	43	73.1	31.8
Spartan	C.A.N. 860	85	0	68	9	51	3	43	5	61.3	4.3
Swanneck	N.S.N. I-33-1	95	1	55	13	61	7	41	21	62.8	10.3
Mean (50 plots)		85.7	2.6	65.8	22.2	59.8	10.7	46.1	16.5		

*C.A.N. = Canadian Accession Number; N.S.N. = University of Alberta Nursery Stock Number.

seed than from seed receiving any of the dehulling treatments. Hand-dehulling proved superior to either scarification or dehulling by acid in inducing infection. The mean percentage of infection of the hulled series was 2.6; of the hand-dehulled, 22.2; of the scarified, 10.7; and of the acid-dehulled, 16.5.

The data were analyzed by the analysis of variance method (7, 10). For this purpose duplicate plots of each variety for each treatment were used, making in all 200. The summarized results are given in Table IV.

TABLE IV

THE ANALYSIS OF VARIANCE FOR SMUT INFECTION

Variations due to	*D/F*	Sum of squares	Mean square	Standard deviation	½ log *e* of mean square	*Z*
Varieties	24	21227.73	884.49	—	3.39	1.58*
Treatments	3	10582.02	3527.34	—	4.08	2.50*
Varieties × treatments	72	11542.23	160.31	—	2.54	0.73*
Replicates†	4	218.52	54.63	—	2.00	0.19**
Error	96	3605.48	37.56	6.13	1.81	
Total	199	47175.98				

**Value of Z exceeds the 1% point.*
***Variance not significant.*
†*Degrees of freedom include 1 for replicates and 3 for interaction replicates × treatments.*

It will be noted that no significant variation due to replicates was found. This indicates that, while the plots had been arranged in a systematic rather than a randomized order, the significance of the results is little affected. It would appear that soil heterogeneity is not an important factor influencing infection from seed-borne pathogens as exemplified by *U. hordei.* Hence randomization of the plots is not essential for a study of this character. Consequently the method of analysis of variance is applicable to the data being presented. The degree of association between infection percentages shown by varieties contained in the duplicate rows of each treatment is given by correlations 1, 2, 3 and 4 (Table VI). It is evident that comparatively high correlations exist between replicates of varieties grown from seed receiving any one of the three dehulling treatments, while one of considerably lower value exists in the case of hulled seed. The agreement was especially high in the hand-dehulled seed where $r = 0.853$. The corresponding values for the scarified and acid-dehulled series were $r = 0.706$ and $r = 0.675$ respectively. These high correlations between replicates of varieties grown from dehulled seed clearly indicate the high agreement between replicates, and reflect favorably on the significance of the data obtained. The correlation coefficient of 0.476, obtained between duplicate values of the hulled series, while mathematically significant in the light of its P value, is of little value in this study owing to the low infection percentages obtained. The magnitude of the correlation value in question was largely determined by

the infection percentage derived from only four of the 25 varieties tested. The same criticism probably applies to all correlation values involving the infection percentages obtained from hulled seed.

The mean square for varieties was highly significant, indicating that the average infections of the varieties tested were significantly different in all tests. A detailed discussion of varietal reaction will be given in a later section of this paper.

The mean square for treatment was also found to be significantly higher than that for error, showing that the average infection percentages induced by the different treatments differed significantly. The standard error of the difference between two 50-plot means would be $6.13 \sqrt{2} / \sqrt{50}$ or 1.23% smut infection. Accepting twice the standard error of the difference as a convenient minimum level of significance, it follows that when the difference between two means exceeds 2×1.23, or 2.46% infection, the chances are greater than 19 : 1 that this difference was not due to chance. From Table III it may be seen that the mean infection resulting from each treatment was as follows: hulled—2.6%; hand-dehulled—22.2%; scarified—10.7%; acid-dehulled—16.5%. It is evident that all four treatments differ significantly in their capacity to induce smut infection.

It has been demonstrated that significant differences in degree of infection exist for both varieties and treatments. The question now arises whether or not these varieties responded similarly in all treatments. The significant mean square of interaction of varieties and treatments shows that some varieties acted in a differential manner in certain of the treatments. A rough comparison of the relative response of the different varieties to the different treatments may be obtained by correlating the average infection percentages of the varieties of one treatment with those of another (correlations 5, 6, 7, 8, 9 and 10, Table VI). The average varietal infections resulting from acid-dehulled seed gave a slightly better agreement with those occurring from hand-dehulled seed than did the corresponding values induced by scarification. In the first instance $r = 0.854$ and in the second, $r = 0.748$. The comparable value between hulled and hand-dehulled seed was considerably lower, r being 0.522.

Effect of dehulling on stand of plants. The average percentage stands of the varieties grown from hulled and dehulled seed are given in Table III. As far as could be observed, the final stand of any variety was a direct reflection of its emergence; all emerged seedlings reaching maturity. It is evident from the data in Table III that the highest stands resulted from hulled seed and the lowest from acid-dehulled seed. Scarification and hand-dehulling gave stands intermediate in number. The average percentage stand of all varieties from hulled seed was 86%; from hand-dehulled seed, 66%; from scarified seed, 60%; and from acid-dehulled seed, 46%. The analysis of variance method was applied also to the data concerning stand of plants. The results are given in Table V.

It will be noted that again no significant variation exists in the case of replicates, demonstrating that any different arrangement of the plots would have had little influence on the significance of the results obtained. The agreement between replicates of the different treatments in regard to percentage stand is shown by correlations 11, 12, 13 and 14 (Table VI). The best agreement existed in the cases of hand-dehulled and scarified lots where correlation coefficients of 0.705 and 0.692 respectively were obtained. In the two replicates from acid-dehulled seed $r = 0.559$, and in the hulled seed $r = 0.403$.

TABLE V

THE ANALYSIS OF VARIANCE FOR PERCENTAGE STAND OF PLANTS

Variation due to	D/F	Sum of squares	Mean square	Standard deviation	½ log e of mean square	Z
Varieties	24	20514.22	854.76	—	3.38	1.06*
Treatments	3	40679.30	13559.73	—	4.76	2.44*
Varieties × treatments	72	12562.70	174.48	—	2.58	0.26*
Replicates**	4	227.40	56.85	—	—	
Error	96	9910.60	103.24	10.16	2.32	
Total	199	83894.22				

**Value of Z exceeds the 1% point.*

***Degrees of freedom include 1 for replicates and 3 for interaction of replicates × treatments.*

The mean square due to varieties is significantly larger than that due to error, indicating that on the average significant differences exist in the capacity of varieties to emerge following the treatments given. The standard error of the difference between two 8-plot means is $10.16\sqrt{2}/\sqrt{8}$ or 5.08%. Differences in mean stand of 10.16% may be judged significant. From the mean percentage stands of the different varieties for all treatments given in Table III it may be seen, for example, that Atlas shows a significantly higher percentage stand than O.A.C. No. 21, and O.A.C. No. 21 in turn, a significantly higher percentage stand than either Lapland or Manchurian. Similarly, the percentage stand of Glabron is significantly higher than that of Comfort and Newal. On the other hand, the varieties Leiorrhynchum, Regal, Velvet and Wisconsin Barbless No. 38 do not differ significantly in this regard.

Variation due to treatment was also significant. The standard error of the difference between two 50-plot means would be $10.16\sqrt{2}/\sqrt{50}$ or 2.03%. Hence, differences of 4.06% may be considered significant. On this basis (see Table III) the varieties grown from seed receiving each of the four treatments differed significantly with regard to mean percentage stand. Hulled seed gave the highest mean stand, followed by hand-dehulled, scarified and acid-dehulled seed in the order mentioned.

The mean square for the interaction of varieties × treatments was significantly higher than the mean square for error, indicating that the varieties did not respond similarly to all treatments. Simple correlations between

the average percentage stand of the varieties of the different treatments should show the degree of any similarity in response. Correlations 15, 16 and 17 (Table VI) show the associations existing between the average percentage stands of varieties grown from hulled seed and those receiving any one of the dehulling treatments. Since the mean reduction in stand of 14% noted in the case of hulled seed is no more than would be expected from normal field germination, the values obtained have little significance in this discussion.

It will be seen that a small significant correlation exists between the average percentage stand of varieties grown from hand-dehulled and those of varieties grown from scarified seed (correlation 18), while a comparatively high correlation exists in this regard in the case of varieties grown from acid-dehulled and scarified seed (correlation 20). It would seem that certain of the factors responsible for reduction in stand when the kernels are dehulled by hand also operate when the seed is scarified. Similarly, certain mutual factors appear to be operative in reducing stand in the scarified and acid-dehulled series.

A non-significant correlation was obtained between the average percentage stands of varieties grown from hand-dehulled and those grown from acid-dehulled seed (correlation 19). This would indicate either that no mutual factors are operative in reducing stand or that the influence of any such factors are masked by the action of the sulphuric acid.

Since distorted seedlings were found in a number of the rows of all three treatments, it was thought that possibly the removal of the hull unduly predisposed the young seedlings to attack by the smut fungus at a time when they were most susceptible, and consequently resulted in their failure to emerge. Such a condition would explain the small but significant correlation existing between the average percentage stands of hand-dehulled and scarified seeds. Working on the assumption that the degree of seedling injury would be proportional to the infection percentages occurring in the mature plants, correlations were calculated between the average percentage infections and average percentage stands occurring amongst the varieties of each dehulling treatment. A non-significant correlation was found to exist between these variables in the hand-dehulled treatment (correlation 21) while a comparatively high, significant, negative value was obtained in the case of acid-dehulled seed (correlation 23) and one of doubtful significance in the case of scarified seed (correlation 22). The lack of significant correlation between percentage infection and stand of the varieties grown from hand-dehulled seed indicates that, if seedling injury from infection with *U. hordei* is a cause of reduced stand in a given variety, it is not closely associated with the susceptibility of that variety to smut, as determined by final smut percentages in mature plants.

The existence of a negative correlation between average percentage infection and stand of varieties from acid-dehulled seed suggests a direct relation between susceptibility to covered smut and kernel susceptibility to acid injury.

TABLE VI

SIMPLE CORRELATIONS OBTAINED IN THE STUDY OF PERCENTAGE COVERED SMUT INFECTION, PERCENTAGE STAND, AND NUMBER OF DAYS FROM EMERGENCE TO HEADING, OF VARIETIES GROWN FROM HULLED, HAND-DEHULLED, SCARIFIED AND ACID-DEHULLED SEED

Variables correlated	r	P
1. Smut percentages between replicates of varieties grown from hulled seed	0.476	0.02–0.01
2. Smut percentages between replicates of varieties grown from hand-dehulled seed	0.853	<0.01
3. Smut percentages between replicates of varieties grown from scarified seed	0.706	<0.01
4. Smut percentages between replicates of varieties grown from acid-dehulled seed	0.675	<0.01
5. Average smut percentages of varieties grown from hulled and hand-dehulled seed	0.522	<0.01
6. Average smut percentages of varieties grown from hulled and scarified seed	0.689	<0.01
7. Average smut percentages of varieties grown from hulled and acid-dehulled seed	0.363	0.1–0.05
8. Average smut percentages of varieties grown from hand-dehulled and scarified seed	0.748	<0.01
9. Average smut percentages of varieties grown from hand-dehulled and acid-dehulled seed	0.854	<0.01
10. Average smut percentages of varieties grown from scarified and acid-dehulled seed	0.632	<0.01
11. Percentage stand of varieties in two replicates grown from hulled seed	0.403	0.05–0.02
12. Percentage stand of varieties in two replicates grown from hand-dehulled seed	0.705	<0.01
13. Percentage stand of varieties in two replicates grown from scarified seed	0.692	<0.01
14. Percentage stand of varieties in two replicates grown from acid-dehulled seed	0.559	<0.01
15. Average percentage stand of varieties grown from hulled and hand-dehulled seed	0.466	0.02–0.01
16. Average percentage stand of varieties grown from hulled and scarified seed	0.418	0.05–0.02
17. Average percentage stand of varieties grown from hulled and acid-dehulled seed	0.286	0.2–0.1
18. Average percentage stand of varieties grown from hand-dehulled and scarified seed	0.435	0.05–0.02
19. Average percentage stand of varieties grown from hand-dehulled and acid-dehulled seed	0.159	0.5–0.4
20. Average percentage stand of varieties grown from scarified and acid-dehulled seed	0.655	<0.01
21. Average percentage infection and average percentage stand of varieties grown from hand-dehulled seed	0.107	0.6
22. Average percentage infection and average percentage stand of varieties grown from scarified seed	−0.312	0.2–0.1
23. Average percentage infection and average percentage stand of varieties grown from acid-dehulled seed	−0.734	<0.01
24. Average percentage infection (hand-dehulled) and average percentage stand (acid-dehulled)	−0.514	<0.01
25. Average percentage infection (hand-dehulled) and average percentage stand (scarified)	−0.087	0.7–0.6
26. Average number of days to heading and average percentage infection of varieties grown from hand-dehulled seed	0.542	<0.01
27. Average number of days to heading and average percentage infection of varieties grown from scarified seed	0.294	0.3–0.2
28. Average number of days to heading and average percentage infection of varieties grown from acid-dehulled seed	0.600	<0.01
29. Average number of days to heading and average percentage infection of varieties grown from hulled seed	0.169	0.5–0.4

In other words, the acid-susceptible or thin-hulled varieties appear to be more susceptible to smut. It was thought at first that this relation was apparent rather than real. That is, it was supposed that, while the acid treatment caused greater seedling mortality in the case of the thin-hulled or acid-susceptible varieties than it did in the case of the thick-hulled, it also tended to dehull the former more effectively and to induce a correspondingly higher infection. This condition also seemed to exist in the case of varieties scarified with sandpaper (correlation 22). The correlation value $r = -0.312$ obtained between average percentage stand and average percentage infection of the varieties receiving this treatment, while of doubtful significance in the light of its P value, 0.1, suggests that the thin-hulled varieties not only suffer greater seedling mortality from scarification than the thick, but also appear to be more effectively dehulled. This explanation, however, failed to hold true when it was found that a negative correlation coefficient of −0.514 also existed between average percentage infection of varieties grown from hand-dehulled seed and average percentage stand of the varieties grown from acid-dehulled seed (correlation 24). Obviously in this case the infection

TABLE VII

AVERAGE PERCENTAGE STAND AND SMUT INFECTION, AND AMOUNT OF HULL REMAINING ON KERNELS OF VARIETIES GROWN FROM ACID-DEHULLED SEED IN 1934

Variety	Number*	Amount of hull remaining over		Average percentage stand	Average percentage infection
		Embryo	Endosperm		
Lapland	N.S.N. I-32-2	very thin to absent	absent	17	32
Canadian Thorpe	C.A.N. 816	very thin to absent	absent	32	40
Hannchen	C.A.N. 837	very thin to absent	absent	41	43
Manchurian	C.A.N. 726	thin to absent	absent	15	46
Peatland	C.A.N. 722	thin to absent	absent	23	16
Shaw	C.A.N. 1047	thin to absent	absent	27	14
Bearer	C.A.N. 704	thin to absent	very thin to absent	49	28
Binder	N.S.N. I-32-8	thin to absent	very thin to absent	47	20
Gold	C.A.N. 829	thin to absent	thin to absent	33	49
Colsess	C.A.N. 772	thin to very thin	thin to absent	33	33
O.A.C. No. 21	C.A.N. 734	thin	very thin	41	4
Sol	C.A.N. 782	thin	very thin	39	4
Spartan	C.A.N. 860	moderately thick to thin	thin to absent	43	5
Swanneck	N.S.N. I-33-1	moderately thick to thin	thin to absent	41	21
Atlas	C.A.N. 702	moderately thick	thin	75	1
Comfort	C.A.N. 712	moderately thick	thin	61	6
Duckbill	C.A.N. 826	moderately thick	thin	57	4
Glabron	C.A.N. 718	moderately thick	thin	67	1
Leiorrhynchum	N.S.N. I-32-11	moderately thick	thin	68	4
Newal	C.A.N. 1089	moderately thick	thin	56	7
Regal	C.A.N. 742	moderately thick	thin	57	8
Trebi	C.A.N. 753	moderately thick	thin	63	21
Vaughn	C.A.N. 759	moderately thick	thin	57	8
Velvet	C.A.N. 755	moderately thick	thin	55	3
Wisconsin Barbless No. 38	C.A.N. 758	moderately thick	thin	60	0

* *C.A.N. = Canadian Accession Number; N.S.N. = University of Alberta Nursery Stock Number.*

percentages obtained are entirely independent of degree of dehulling. This result shows that the relation between smut infection and kernel susceptibility to acid injury is in part a real one. Varieties showing the greatest loss of stand from acid treatment tend to be inherently more susceptible to covered smut. This conclusion does not apply in the case of varieties grown from scarified seed. A non-significant correlation was obtained between smut percentages induced by hand-dehulled seed and percentage stand resulting from scarification (correlation 25).

Further and more detailed data regarding the susceptibility of the kernels of different varieties to acid injury are given in Table VII. The varieties have been listed according to the amount of hull remaining on the kernel following treatment with sulphuric acid. The corresponding average percentage stands and smut infections are also given.

It will be noted that considerable varietal differences exist either in thickness of kernel hull or in its resistance to decomposition by acid. Varieties possessing thin hulls over the embryo after acid treatment tend to show low emergence percentages and generally high infection percentages (See Hannchen and Canadian Thorpe, Fig. 1). The opposite condition tends to prevail in the

FIG. 1. *Stand of barley varieties from seed dehulled with concentrated sulphuric acid followed by inoculation with chlamydospores of Ustilago hordei, Edmonton, 1934.*
Left to right: Hannchen, Canadian Thorpe, Leiorrhynchum, Newal, Wisconsin Barbless No. 38, Comfort, Regal, Velvet and Glabron.

case of varieties possessing moderately thick hulls after acid treatment. However, one or two exceptions occur. O.A.C. No. 21 and Sol, while showing thin hulls after acid treatment, exhibit considerable resistance to smut, which is probably physiological resistance. On the other hand, Trebi shows considerable susceptibility to smut in spite of the fact that it possesses a moderately thick or acid-resistant hull. It is interesting to note that the smooth-awned varieties all show moderately thick hulls over the embryo, after acid treatment, and these varieties produced the best stands (Fig. 1).

It would be of interest at this time to determine statistically the particular susceptibility to injury shown by certain varieties after being given the different treatments. Such a determination involves the use of cross differences. The methods involved have been clearly set out by Immer, Hayes and Powers (10).

In Table VIII are given the deviations of percentage stand of two plots of the varieties grown from seed receiving a given treatment, from the average percentage stand of the same variety grown from seed receiving the other three treatments, minus the difference between the average percentage stand of all varieties grown from seed receiving that treatment and the average percentage stand of all varieties grown from seed receiving the other three treatments. These differences express the degree of the increase in percentage stand of each variety grown from seed receiving each treatment, over the percentage stand shown by varieties grown from seed receiving the other three treatments, aside from the general increase or decrease of that treatment over the others. Plus deviations show a response more favorable and negative deviations a response less favorable than the average.

Cross differences are the differences between any two deviations in Table VIII. The error of the cross differences is calculated in the following manner: The standard error of a single total of two plots per variety per treatment

TABLE VIII

DEVIATIONS OF PERCENTAGE STANDS OF TWO PLOTS OF THE VARIETIES GROWN FROM SEED RECEIVING A GIVEN TREATMENT FROM THE AVERAGE PERCENTAGE STAND OF THE SAME VARIETY GROWN FROM SEED RECEIVING THE THREE OTHER TREATMENTS, MINUS THE DIFFERENCE BETWEEN THE AVERAGE PERCENTAGE STAND OF ALL VARIETIES GROWN FROM SEED RECEIVING THAT TREATMENT AND THE AVERAGE PERCENTAGE STAND OF ALL VARIETIES GROWN FROM SEED RECEIVING THE OTHER THREE TREATMENTS

Variety	Treatment			
	Hulled	Hand-dehulled	Scarified	Acid-dehulled
Atlas	−43.99	− 8.14	22.36	29.77
Bearer	− 6.32	− 3.81	− 6.64	20.56
Binder	− 5.99	8.53	15.02	−17.56
Canadian Thorpe	8.35	37.53	−11.98	−33.89
Comfort	− 4.32	− 4.47	−39.31	48.11
Colsess	− 6.99	12.86	23.36	−29.23
Duckbill	− 9.99	7.19	− 3.64	6.44
Glabron	− 7.32	− 7.47	− 6.31	21.11
Gold	− 1.99	32.53	0.36	−30.89
Hannchen	−22.65	30.53	27.69	−35.56
Lapland	22.01	− 7 47	− 2.31	−12.23
Leiorrhynchum	− 2.65	−81.47	37.02	47.11
Manchurian	59.35	0.53	−14.31	−45.56
Newal	24.68	−67.47	4.36	38.44
O.A.C. No. 21	3.68	30.19	−15.31	−18.56
Peatland	12.68	45.86	−12.98	−45.56
Regal	−19.32	− 0.81	− 2.31	22.44
Shaw	23.01	−11.81	10.69	−21.89
Sol	10.68	− 1.47	−22.98	13.77
Spartan	6.35	14.19	−17.98	− 2.56
Swanneck	27.68	−25.81	8.69	−10.56
Trebi	−36.32	0.86	18.02	17.44
Vaughn	−12.65	−14.14	− 0.98	27.77
Velvet	5.35	3.86	−24.31	15.11
Wisconsin Barbless No. 38	−32.65	0.53	4.36	27.77

is $10.16\sqrt{2}$ or 14.37. The standard error of the difference between one such total and the average of three others is $\sqrt{(14.37)^2 + \frac{(14.37)^2}{3}}$ or 16.58. The standard error of the difference between two such differences would be $16.58\sqrt{2}$ or 23.45. Cross differences in excess of 46.9 may be judged significant. With reference to deviations obtained from acid-dehulled seed, it will be seen the varieties Peatland, Manchurian, Hannchen, Canadian Thorpe, Gold, Colsess, Shaw and O.A.C. No. 21 appear to be particularly susceptible to acid injury. Of these, Peatland, Manchurian, Hannchen and Canadian Thorpe responded in a differential manner when compared with any one of the varieties giving plus deviations, with the exception of Duckbill. The smooth-awned varieties generally gave good plus deviations, indicating a response more favorable than the average. These data agree in a general way with those given in Table VII.

Comfort, Velvet and Sol appear to be particularly susceptible to injury from scarification. Comfort shows a differential response when compared with the varieties Atlas, Binder, Hannchen, Leiorrhynchum and Trebi. The two varieties, Leiorrhynchum and Newal showed particularly low stands from the hand-dehulled treatment. They both show differential response when compared with all varieties possessing plus deviations. It will be noted also that Duckbill responded uniformly to all treatments. O.A.C. No. 21 appears to be equally susceptible to injury from either scarification or acid-dehulling.

Effect of dehulling on earliness of heading. The varieties grown from acid-dehulled and scarified seed were found to be generally delayed in heading by 1½ and 2½ days respectively, as compared with those grown from either hulled or hand-dehulled seed. This retardation was thought to be due to slow initial growth caused by either mutilation of the embryo or loss of endosperm.

In connection with earliness of heading it is of interest to point out that a correlation value of $r = 0.542$ exists between average percentage infection and average number of days from emergence to heading in the case of the varieties grown from hand-dehulled seed (correlation 26). This indicates that the later-maturing varieties tend to be more susceptible to covered smut. A significant correlation also existed between these variables when acid-dehulled seed was used (correlation 28), but the correlation values were not significant in the cases of scarified and hulled seed (correlations 27 and 29). Low infection percentages undoubtedly explain the non-significant correlation found when hulled seed was used while the delayed development caused by scarification possibly obscured any association between date of heading and percentage smut infection.

Varietal resistance. In Table IX are given the eight-plot total and mean infection percentages of the varieties tested, together with the appropriate standard errors of the difference. The varieties are arranged in order of susceptibility. It will be seen that infection percentages range from a mean

of over 30% for the varieties Manchurian and Hannchen, to a mean of less than 1% for Atlas and Wisconsin Barbless No. 38. Lapland and Gold show mean infection percentages of over 25%; Canadian Thorpe, Colsess and Binder, mean infection percentages between 20 and 25%; and Trebi between 15 and 20%.

TABLE IX

TOTAL AND MEAN PERCENTAGE COVERED SMUT INFECTION OF EIGHT PLOTS OF EACH VARIETY

Variety	Percentage infection	
	Total	Mean
Manchurian	268	33.5
Hannchen	254	31.8
Lapland	237	29.6
Gold	220	27.5
Canadian Thorpe	196	24.5
Colsess	195	24.4
Binder	161	20.1
Trebi	145	18.1
Duckbill	117	14.6
Bearer	115	14.4
Peatland	113	14.1
Newal	106	13.3
Swanneck	82	10.3
Shaw	68	8.5
Comfort	61	7.6
Sol	54	6.8
Regal	51	6.4
Vaughn	34	4.3
Spartan	34	4.3
Glabron	24	3.0
Leirrohynchum	22	2.8
Velvet	18	2.3
O.A.C. No. 21	17	2.1
Atlas	4	0.5
Wisconsin Barbless No. 38	2	0.3
Standard error of difference	24.51	3.06

Differences between two means, exceeding twice the standard error of the difference or 6.12%, may be considered significant. It is evident that Manchurian, Hannchen, Lapland and Gold do not differ significantly in mean percentage infection. Similarly the more resistant varieties listed below Regal do not differ significantly. On the other hand, using only a few examples, Hannchen shows significantly higher mean infection percentage than Canadian Thorpe or Colsess, and Trebi a significantly higher mean infection percentage than those varieties listed below Newal.

The discussion of varietal resistance would be more clear if the varieties were classified into several commonly recognized groups. In Table III the varieties have been arranged in such a manner. The eight-plot means of each variety are given in the column to the right of the table.

Six-rowed, hulled, rough-awned types. High resistance to covered smut is shown by three varieties of this group; namely, Atlas, O.A.C. No. 21 and Vaughn. These varieties are all significantly lower in mean percentage infection than any one of the other five varieties of this group. Manchurian and Lapland evidence high susceptibility and do not differ significantly in this regard. They both show, however, significant increases over Bearer, Trebi and Peatland, varieties possessing moderate susceptibility.

Six-rowed, hulled, smooth-awned types. This group of barleys, with the exception of Newal and possibly Comfort, possesses a general resistance to covered smut. It will be noted that, while Comfort has a fairly low mean

percentage infection, it averaged 21% when grown from hand-dehulled seed. Newal shows significantly higher mean percentage smut infection than all other varieties with the exception of Comfort. Both Comfort and Regal possess significant increases over Wisconsin Barbless No. 38.

Six-rowed, hulled, hooded types. Of the three varieties comprising this group, Shaw and Sol exhibited moderate resistance while Colsess showed considerable susceptibility. Colsess significantly exceeded both Shaw and Sol with regard to mean percentage infection. Shaw and Sol did not differ significantly in this regard.

Two-rowed, hulled types. High susceptibility to covered smut is evidenced by Hannchen, Gold, Binder and Canadian Thorpe. Duckbill appeared moderately susceptible, and Spartan and Swanneck moderately resistant. Hannchen and Gold do not differ significantly with regard to mean infection percentages, but both exceed the varieties Binder, Duckbill, Swanneck and Spartan in this regard. Canadian Thorpe is exceeded significantly in mean infection percentage by Hannchen only.

The smut reactions of the varieties in the 1934 test grown from either hand-dehulled or acid-dehulled seed agreed very well with those grown from acid-dehulled seed in 1932. This conclusion is based on the reaction of 18 varieties. Values of $r = 0.811$ ($P = <.01$) and $r = 0.614$ ($P = <.01$) were obtained between the smut percentages obtained in 1932 from the Edmonton collection of smut and the average of those obtained in 1934 from hand-dehulled and acid-dehulled seed respectively. When the smut percentages obtained in 1932 from the Edmonton and Winterburn collections were averaged, the values were 0.628 ($P = <.01$) and 0.526 ($P = <.01$) respectively.

Physiologic Specialization

Literature Review

Faris (5) showed that one requirement necessary to secure high infection percentages on Hannchen and Nepal barleys, was the use of inoculum gathered from those varieties. Hannchen produced 72% of smut when inoculated with chlamydospores from Hannchen, but exhibited only from 1–2% when inoculated with smut from other sources. In a later paper (6), Faris reports the existence of five physiologic forms of covered smut based on their reactions on Nepal, Hannchen, Summit and Texas Winter barleys. Rodenhiser (14) differentiated seven physiologic forms of *U. hordei* in culture based on color, topography, surface, consistency, type of margin of colonies and chemical affinities. These forms were present in collections obtained from various localities in Minnesota, from neighboring states and foreign countries. Two of these forms, one obtained from Italy and the other from Minnesota, were shown to differ in pathogenicity. The variety Himalaya proved resistant to the Italian form and susceptible to the Minnesota form, while the variety Lion, which had hitherto shown immunity to all Minnesota smut collections, displayed susceptibility to the Italian form.

Methods and Experimental Results

An experiment was conducted at the University of Alberta in 1931, in which a test was made of the reaction of eleven barley varieties to six collections of smut. The inoculum was gathered from six points in central Alberta. Separate inoculations of all eleven varieties were made with each of the six smut collections. Seventy-five seeds were sown in duplicate ten-foot rows. The seed of the hulled varieties was not dehulled. The results of this test are summarized in Table X.

The data show that at least two of the six smut collections used are distinct physiologic forms. The collections in question are those obtained from Edmonton and Lacombe. The form from Edmonton is readily distinguished by its reaction on Eureka and Hannchen or Canadian Thorpe. Eureka showed an average of 37% infection when inoculated with the Edmonton collection, but was free from smutted plants when inoculated with any of the other five collections. Canadian Thorpe and Hannchen also proved susceptible to this collection, showing 15 and 20% average infection respectively.

TABLE X

Reaction of eleven varieties of barley to infection with six collections of *Ustilago hordei*, as determined by field tests at the University of Alberta, Edmonton, in 1931

Variety	Canadian accession number	Source of inoculum and percentage of plants smutted																	
		Camrose			Edmonton*			Lacombe			Morinville			Vermilion			Wetaskiwin		
		Replicate			Replicate			Replicate			Replicate			Replicate			Replicate		
		1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.
Canadian Thorpe	816	4	3	4	20	10	15	20	24	22	5	4	5	2	0	1	12	6	9
Colsess	772	3	1	2	2	2	2	10	2	6	6	12	9	2	2	2	0	5	3
Duckbill	826	0	0	0	3	1	2	3	1	2	1	1	1	0	0	0	1	0	1
Eureka	773	0	0	0	40	34	37	0	0	0	0	0	0	0	0	0	0	0	0
Glabron	718	0	1	1	0	2	1	0	0	0	0	0	0	0	2	1	0	0	0
Hannchen	837	3	1	2	20	20	20	6	15	11	5	6	6	6	5	6	8	4	6
O.A.C. No. 21	734	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Peatland	722	13	6	10	2	8	5	9	8	9	5	6	6	1	5	3	8	1	5
Regal	742	2	1	2	1	0	1	1	4	3	1	1	1	1	3	2	1	0	1
Spartan	860	1	0	1	0	0	0	1	3	2	1	1	1	0	4	2	2	1	2
Trebi	753	3	11	7	5	3	4	6	4	5	0	3	2	1	1	1	2	3	3

**This collection of smut was obtained from the variety Success, grown at the University of Alberta.*

The physiologic form represented by the collection of smut from Lacombe is easily distinguished from the Edmonton form by its failure to infect the variety Eureka and the comparatively high percentage of smut it produced on Canadian Thorpe. The collections from Camrose, Morinville, Vermilion and Wetaskiwin do not appear to be sufficiently different from the Lacombe collection to be considered as separate forms. The Vermilion collection produced a low percentage of smutted plants on Canadian Thorpe in contrast

to the Lacombe collection, but this may be due to a lower degree of spore viability. This is indicated by the low percentage of plants infected in all the varieties tested with this collection.

The reliability of the data obtained in this experiment is well illustrated in the high degree of correlation between the infection percentages of the different varieties in the two replicates. The correlation coefficient between the percentage infection in the two replicates of the 11 varieties with the six collections of smut is $+0.832$ ($P = <0.01$). In many instances there was no infection on either replicate. When the correlation coefficient is calculated, using the infection percentages from only those plots in which there was some infection, it is $+0.870$ ($P = <0.01$).

It will be noted in Table X that no smutted plants appeared in any of the rows of O.A.C. No. 21. Regal, Duckbill, Glabron and Spartan appeared highly resistant to all the collections of smut used in this test.

Discussion

There is an urgent need for improved methods for the testing of barley varietal reaction to the covered smut disease. The use of hulled seed has not proved satisfactory, several workers having reported failure to differentiate between susceptible and resistant sorts owing to the low infection percentages obtained (1, 2, 14, 15). Dehulling of the kernels prior to inoculation has resulted in increased infections (1, 6, 17). However, no really satisfactory method of dehulling has yet been reported. Dehulling by hand is laborious and impracticable when large populations are involved. The use of sulphuric acid as suggested by Briggs (1) did not prove altogether satisfactory when used in the concentrated form, by one of the authors, to dehull kernel lots of hybrid material (11). However, the possibilities of this chemical have by no means been exhausted. The feasibility of scarification or some other type of mechanical injury of the seed coat as a means of dehulling has received little attention.

From the point of view of the plant breeder, methods of dehulling that will give reliable and high infections consistent with ease of application are desirable. In varietal testing where only comparative reaction is desired, it is not imperative to have methods giving the highest infections provided that the methods adopted induce infections sufficiently high to allow of the separation of resistant and susceptible sorts.

From the results of the present study, it is evident that the infection percentages obtained from hulled seed are too low and too unreliable to be of value in testing varietal reaction to the covered smut disease. Dehulling by hand, acid or scarification gave significant increases in smut infection. Highest and most reliable infection percentages were obtained from hand-dehulled seed. However, this method involved the greatest time and labor. Acid-dehulled seed gave higher infection percentages than scarified seed, but exhibited considerably more seedling injury as shown by reduced stands.

Acid-dehulling involved the least time and labor of the three methods tested. It must be borne in mind, however, that since seed free from mechanical injury is essential for the success of this treatment, considerable work of a preliminary nature may be necessary in selecting sound seed. The greatest criticism which may be directed at sulphuric acid as a dehulling agent is the seedling injury resulting from its use in the case of certain varieties, which tends to detract somewhat from the significance of the results obtained. Increasing the number of dehulled seeds for the test to at least 100 should increase the reliability of the results obtained by this method.

Scarified seed gave stands only slightly more reduced than those from hand-dehulled seed. However, the agreement of infection percentages induced by scarification with those induced by hand-dehulling was poor in the case of certain varieties. Bearer and Comfort gave 26 and 21% smut infection respectively when grown from hand-dehulled seed and only three and two per cent when grown from scarified seed. Similarly, Hannchen and Gold showed considerably less susceptibility when grown from scarified rather than from hand-dehulled seed. As good agreement exists between replicates, it would appear that scarification as practised in this investigation is ineffective in inducing infection within certain varieties. Generally, however, this method differentiated fairly well between susceptible and resistant varieties. It is felt that, with further investigation, scarification of barley seed as a means of inducing smut infection will prove to be of considerable value in the testing for varietal reaction.

It appears evident, from the data presented, that hand-dehulling should be practised when maximum infections are desired. Removal of the complete hull is not necessary. In the present work only the embryo was exposed and high infection percentages were obtained.

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A COMPARISON OF VARIOUS HARVESTING METHODS IN RESPECT TO MOISTURE CONTENT AND GRADE OF THE GRAIN[1]

(FINAL REPORT)

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Abstract

In a moisture and grade survey of grain harvested by various methods in parts of Manitoba and Saskatchewan in 1933, it was found that straight-combined wheat showed a much greater percentage of tough and damp samples than either stook-threshed or swath-combined samples. Of 246 straight-combined samples, 18% were tough and 7% damp; of 212 stook-threshed samples, 10% were tough and none damp; of 184 swath-combined samples 6.5% were tough and 0.5% were damp. These results confirm those obtained in 1932.

In common wheat there was a marked decrease in grade between the early- and late-season samples. Average grades were 1.37, 1.32 and 1.28 for stook-threshed, straight-combined and swath-combined samples respectively. This is quite the reverse of the order found in 1932. Taking the two years' samples, collectively, the average grades are 1.18, 1.28 and 1.36 for the methods in the order given above. In view of the conflicting results for the two consecutive seasons, no definite conclusion can be drawn with regard to the average grade of common wheat threshed by these three methods.

With durum wheat in 1933 the average grades were 1.29, 1.79 and 2.00 for stook-threshed, straight-combined and swath-combined samples respectively. Differentiation of the three harvesting methods on basis of grade of durum wheat was greater in 1933 than in 1932. This points firmly to the conclusion that stook threshing is the best method for this class of wheat in Manitoba.

A small series of 32 barley samples collected in Manitoba in 1933 showed no differentiation in moisture content as a result of method of harvesting. However, stook-threshed samples of barley tended to grade higher than those threshed by either of the combine methods.

In an earlier paper Larmour, Geddes and Cameron (1) discussed the conditions which led to the investigation of variation in moisture and grade of grain harvested in different ways. While the survey of 1932 was fairly comprehensive in covering the general areas of Western Canada in which combine harvesting is practised, it was considered advisable to conduct a similar survey in the following season in order to avoid the dangers in conclusion that are liable to be made when depending on only one year's data. This paper is a report of the observations on samples collected in 1933 and a general summary of the results of the whole project.

Owing to the conditions of extreme drought prevailing in 1933 in central and southern Saskatchewan and Alberta, the number of samples was reduced considerably. No field samples were collected in Alberta because practically all the normal combine area in that province was severely affected by drought.

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In Saskatchewan, the areas of greatest combine concentration, namely the Rosetown-Kindersley district, had almost a complete failure, but the crop was fairly good in the middle-south of the province and most of the sampling in 1933 was done in this area. In Manitoba the area covered was approximately the same as in the preceding year. Collection of samples and determination of moisture and grades were carried out as in 1932.

As in the previous work (1) the collections were divided into three parts: (*i*) the early harvest season, before any rain had occurred, (*ii*) a short period after one general rain and (*iii*) the later harvest season after two or more rains had occurred. The numbers of samples collected in these periods are given in Table I.

TABLE I

CLASSIFICATION OF SAMPLES ACCORDING TO METHOD OF HARVESTING, EXPOSURE TO RAINFALL AND ORIGIN, 1933

	Before rain		After one rain		After two or more rains		Grand total	
	No.	%	No.	%	No.	%	No.	%
Stook-threshed—								
Manitoba common	61	9.5	17	2.6	9	1.4	87	13.6
Manitoba durum	15	2.3	6	0.9	43	6.7	64	10.0
Saskatchewan	10	1.6	4	0.6	47	7.3	61	9.5
Total	86	13.4	27	4.1	99	15.4	212	33.1
Straight-combined—								
Manitoba common	20	3.1	2	0.3	–	–	22	3.4
Manitoba durum	15	2.3	2	0.3	13	2.0	30	4.7
Saskatchewan	135	21.0	13	2.0	46	7.2	194	30.2
Total	170	26.4	17	2.6	59	9.2	246	38.3
Swath-combined—								
Manitoba common	47	7.3	14	2.2	7	1.1	68	10.6
Manitoba durum	28	4.4	6	0.9	44	6.8	78	12.1
Saskatchewan	17	2.6	2	0.3	19	3.0	38	5.9
Total	92	14.3	22	3.4	70	10.9	184	28.6
Grand totals, all methods	348	54.2	66	10.3	228	35.5	642	100.0

The total number of samples collected was 642 as compared with 1028 in the 1932 season. Of these 33% were stook-threshed, 38% straight-combined and 29% swath-combined: 54% were collected before any rain, 10% after one rain and 36% after two or more rains.

Moisture in Wheat Samples Harvested Before Rain

A summary of the distribution of moisture in this class is given in Table II. All the stook-threshed samples were dry; of the 170 straight combined samples, 14% were tough and 8% were damp; of the 82 swath-combined samples 4% were tough and all the rest dry. *These results confirm those of the preceding*

TABLE II

DISTRIBUTION OF MOISTURE IN WHEAT SAMPLES HARVESTED BEFORE ANY RAIN, 1933. COMMON AND DURUM WHEAT

Moisture range, %	Stook-threshed					Straight-combined					Swath-combined				
	Man.	Sask.	Total	%	Average moisture	Man.	Sask.	Total	%	Average moisture	Man.	Sask.	Total	%	Average moisture
Straight grade—															
<10.9	27	4	31	36.1	10.4	3	30	33	19.4	10.4	26	8	34	37.0	10.2
10.9–11.7	18	3	21	24.4	11.2	9	28	37	21.8	11.3	18	5	23	25.0	11.2
11.8–12.6	16	2	18	20.9	12.1	9	20	29	17.1	12.1	13	1	14	15.2	12.2
12.7–13.5	10	1	11	12.8	12.9	6	18	24	14.1	13.0	10	3	13	14.1	13.0
13.6–14.4	5	—	5	5.8	14.0	0	9	9	5.3	14.0	4	—	4	4.3	14.0
Total			86	100.0				132	77.7				88	95.6	
Tough—															
14.5–15.3	—	—	—	—	—	2	8	10	5.9	15.0	3	—	3	3.3	14.8
15.4–16.2	—	—	—	—	—	—	2	2	1.2	15.9	1	—	1	1.1	15.8
16.3–17.0	—	—	—	—	—	3	9	12	7.0	16.6	—	—	—	—	—
Total								24	14.1				4	4.4	
Damp—															
17.1–17.9	—	—	—	—	—	—	6	6	3.5	17.6	—	—	—	—	—
18.0–18.8	—	—	—	—	—	3	1	4	2.4	18.4	—	—	—	—	—
> 18.9	—	—	—	—	—	—	4	4	2.3	22.1	—	—	—	—	—
Total								14	8.2						
Total in each group			86	100.0				170	100.0				92	100.0	
Total in series								348							

year, when in a similar group 98% of both the stook-threshed and swath-combined samples were below 14.5% moisture, while the straight combined samples showed 16% tough and damp compared with 22% for 1933. Again it must be concluded that even under the most favorable harvesting conditions at the beginning of the season, there is a tendency for straight-combine operators to start harvesting too early.

Samples Collected After One Rain

This group was relatively larger than in the previous season, consisting of 66 samples or 10% of the total, as compared with 77 or 7.5% of the total in 1932. The data in Table III show that 15% of the stook-threshed, 12% of the straight-combined and none of the swath-combined samples collected in this period were tough; none were damp. This is quite different from the observations for the corresponding period of 1932 when these three methods in the order named gave 13%, 32% and 75% tough and damp samples.

Samples Collected after Two or More Rains

The number of samples in this class in 1933 was 35.5% of the total as compared with 50% for the preceding year. The fall rains were, in general, later in 1933 than in 1932. The grain, therefore, had every chance to ripen and consequently very few of the tough and damp samples could be attributed to immature grain. The data in Table IV show that of the stook-threshed samples 17.2% were tough and none damp; in the corresponding group of 1932 only 3% were tough. The straight-combine method gave 32.2% tough and 5.1% damp; in the previous season there were 25% tough and 3% damp. The swath-combine method showed 11.4% tough and 1.4% damp, compared with 9% and 2% respectively in 1932. On the whole, there was a greater incidence of tough and damp samples in 1933 than in 1932 during the late harvesting period.

The data of Tables II, III and IV are combined in Table V, summarizing the distribution of moisture for all samples collected in 1933. Of 212 stook-threshed samples 10% were tough and none damp; of 246 straight-combined samples 18% were tough and 7% damp; of 184 swath-combined samples 6.5% were tough and 0.5% damp.

Close examination of Table V shows that the degree of toughness did not vary much with the three harvesting methods. The weighted average moistures of the tough samples are 15.4%, 15.5% and 15.3% for stook-threshed, straight-combined and swath-combined respectively. The dampest samples were obtained by the straight-combine method. Of the 18 damp samples in 1933, 17 were straight combined and of these 5 were above 19.7% moisture and averaged 22.1% moisture content. In the straight grade moisture range, the stook-threshed and straight-combined samples show about the same average moistures, while the swath-combined samples tend to be somewhat lower in moisture.

TABLE III

DISTRIBUTION OF MOISTURE IN WHEAT SAMPLES HARVESTED AFTER ONE RAIN, 1933. COMMON AND DURUM WHEAT

Moisture range, %	Stook-threshed					Straight-combined					Swath-combined				
	Man.	Sask.	Total	%	Average moisture	Man.	Sask.	Total	%	Average moisture	Man.	Sask.	Total	%	Average moisture
Straight grade—															
<10.9	6	—	6	22.2	10.4	—	1	1	5.9	10.6	5	—	5	22.7	10.0
10.9–11.7	8	—	8	29.6	11.4	1	2	3	17.6	11.5	7	—	7	31.8	11.2
11.8–12.6	6	1	7	25.9	12.1	—	5	5	29.4	12.3	7	2	9	40.9	12.0
12.7–13.5	—	—	—	—	—	2	3	5	29.4	13.2	1	—	1	4.6	13.5
13.6–14.4	1	1	2	7.4	13.7	1	—	1	5.9	14.4	—	—	—	—	—
Total			23	85.2				15	88.2				22	100.0	
Tough—															
14.5–15.3	1	2	3	11.1	14.8	—	—	—	—	—	—	—	—	—	—
15.4–16.2	1	—	1	3.7	16.2	—	2	2	11.8	15.6	—	—	—	—	—
16.3–17.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total			4	14.8				2	11.8						
Damp—															
17.1–17.9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
18.0–18.8	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
>18.9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total in each group			27	100.0				17	100.0				22	100.0	
Total in series								66							

TABLE IV

DISTRIBUTION OF MOISTURE IN WHEAT SAMPLES HARVESTED AFTER TWO OR MORE RAINS, 1933. COMMON AND DURUM WHEAT

Moisture range, %	Stook-threshed					Straight-combined					Swath-combined				
	Man.	Sask.	Total	%	Average moisture	Man.	Sask.	Total	%	Average moisture	Man.	Sask.	Total	%	Average moisture
Straight grade—															
<10.9	—	—	—	—	—	1	—	1	1.7	10.7	4	—	4	5.7	10.6
10.9–11.7	10	1	11	11.1	11.4	—	2	2	3.4	11.5	13	1	14	20.0	11.4
11.8–12.6	15	5	20	20.2	12.1	2	2	4	6.8	12.4	13	5	18	25.7	12.1
12.7–13.5	16	14	30	30.3	13.1	4	8	12	20.3	13.0	13	7	20	28.6	13.0
13.6–14.4	8	13	21	21.2	14.0	3	15	18	30.5	14.0	1	4	5	7.1	14.0
Total			82	82.8				37	62.7				61	87.1	
Tough—															
14.5–15.3	3	6	9	9.1	14.8	1	11	12	20.3	14.8	1	1	2	2.9	14.6
15.4–16.2	—	5	5	5.1	15.9	1	5	6	10.2	15.8	4	1	5	7.1	15.6
16.3–17.0	—	3	3	3.0	16.6	—	1	1	1.7	16.6	1	—	1	1.4	16.3
Total			17	17.2				19	32.2				8	11.4	
Damp—															
17.1–17.9	—	—	—	—	—	—	2	2	3.4	17.2	—	—	—	—	—
18.0–18.8	—	—	—	—	—	—	—	—	—	—	1	—	1	1.4	18.1
>18.8	—	—	—	—	—	1	—	1	1.7	19.9	—	—	—	—	—
Total								3	5.1				1	1.4	
Total in each group			99	100.0				59	100.0				70	100.0	
Total in series	228														

TABLE V

DISTRIBUTION OF MOISTURE, ALL WHEAT SAMPLES COLLECTED IN 1933

Moisture range, %	Stook-threshed					Straight-combined					Swath-combined				
	Man.	Sask.	Total	%	Average moisture	Man.	Sask.	Total	%	Average moisture	Man.	Sask.	Total	%	Average moisture
Straight grade—															
<10.9	33	4	37	17.5	10.4	4	31	35	14.2	10.4	35	8	43	23.4	10.2
10.9–11.7	36	4	40	18.9	11.3	10	32	42	17.1	11.3	38	6	44	23.9	11.3
11.8–12.6	37	8	45	21.2	12.1	11	27	38	15.4	12.2	33	8	41	22.3	12.1
12.7–13.5	26	15	41	19.3	13.0	12	29	41	16.7	13.0	24	10	34	18.5	13.0
13.6–14.4	14	14	28	13.2	14.0	4	24	28	11.4	14.0	5	4	9	4.9	14.0
Totals			191	90.1				184	74.8				171	93.0	
Tough—															
14.5–15.3	4	8	12	5.7	14.8	3	19	22	8.9	14.9	4	1	5	2.7	14.7
15.4–16.2	1	5	6	2.8	16.0	1	9	10	4.1	15.8	5	1	6	3.3	15.6
16.3–17.0	—	3	3	1.4	16.6	3	10	13	5.3	16.6	1	—	1	0.5	16.3
Totals			21	9.9				45	18.3				12	6.5	
Damp—															
17.1–17.9	—	—	—	—	—	—	8	8	3.2	17.5	—	—	—	—	—
18.0–18.8	—	—	—	—	—	3	1	4	1.6	18.4	—	—	—	—	—
18.9–19.7	—	—	—	—	—	—	—	—	—	—	1	—	1	0.3	19.1
> 19.7	—	—	—	—	—	1	4	5	2.0	22.1	—	—	—	—	—
Totals								17	6.9				1	0.5	
Grand totals			212	100.0				246	100.0				184	100.0	
Total in series	642														

Comparison of Results of 1932 and 1933

A summary of the data obtained in 1932 and 1933 and of the combined data for the two years is given in Table VI, in which the percentages of samples higher than 14.4% in moisture content are shown. The greatest variations are found in the straight-combined samples in the three groupings, "before rain", "after one rain" and "after two or more rains". These differences disappear in the results for the whole season; the 1932 and 1933 collections each showed 25% tough and damp samples by this method. The swath-combined samples had 9% and 7% tough and damp for 1932 and 1933 respectively, with 8% for the two years combined. The greatest difference between the two years occurred in the stook-threshed samples, in which there were 3% and 10% tough and damp for 1932 and 1933 respectively. This was due to the high percentage of tough samples in the late season collection in 1933.

TABLE VI

PERCENTAGE OF SAMPLES HIGHER THAN 14.4% IN MOISTURE CONTENT IN THE 1932, 1933 AND COMBINED COLLECTIONS

	Before rain, %	After one rain, %	After two or more rains, %	All samples, %
Stook-threshed—				
1932	3	13	3	3
1933	0	15	17	10
Collectively	1	14	8.4	5.4
Straight-combined—				
1932	19	34	28	25
1933	38	12	37	25
Collectively	19.5	28	30	25
Swath-combined—				
1932	2	75*	11	9
1933	4	0	13	7
Collectively	3	20	12.4	8

*This percentage was based on only 8 samples.

The Relation of Harvesting Method to the Grade of the Sample

Common Wheat

The official grades of all common wheat samples collected in 1933 are given in Table VII. The average grade was calculated as described by Larmour, Geddes and Cameron (1) by assigning the arbitrary values, 0, 1, 2, 3, 4, 5, 3, 4, 5 and 6 to grades 1 Hard, Nos. 1, 2, 3 and 4 Northern, No. 5, No. 1 Nor. rejected, No. 2 Nor. rejected, No. 3 Nor. rejected and No. 4 Nor. rejected, respectively. Thus the smaller the figure, the higher is the average grade.

TABLE VII

DISTRIBUTION OF SAMPLES ACCORDING TO GRADE—COMMON WHEAT 1933

Grade	Stook-threshed				Straight-combined				Swath-combined			
	Man.	Sask.	Total	%	Man	Sask.	Total	%	Man.	Sask.	Total	%
Samples collected before rain												
1 Hard	6	3	9	12.7	—	26	26	16.8	3	11	14	21.9
1 Northern	46	5	51	71.8	16	68	84	54.2	34	3	37	57.8
2 Northern	9	1	10	14.1	3	33	36	23.2	7	3	10	15.6
3 Northern	—	—	—	—	1	6	7	4.5	3	—	3	4.7
4 Northern	—	—	—	—	—	1(1)	1	0.7	—	—	—	—
No. 5	—	1	1	1.4	—	1(1)	1	0.7	—	—	—	—
Total	61	10	71		20	135	155		47	17	64	
Average grade	1.07				1.20				1.00			
Samples collected after one rain												
1 Hard	2	1	3	14.3	—	1	1	6.7	—	—	—	—
1 Northern	13	1	14	66.7	2	11	13	86.7	6	1	7	43.8
2 Northern	2	1	3	14.3	—	1	1	6.7	8	1	9	56.2
3 Northern	—	1	1	4.7	—	—	—	—	—	—	—	—
Total	17	4	21		2	13	15		14	2	16	
Average grade	1.10				1.0				1.56			
Samples collected after two rains												
1 Hard	—	1	1	1.8	—	—	—	—	—	—	—	—
1 Northern	4	13	17	30.9	—	10	10	21.7	1	2	3	11.3
2 Northern	5	23	28	50.9	—	34	34	73.9	1	16	17	65.4
3 Northern	—	7	7	12.7	—	2	2	4.4	5	1	6	23.1
4 Northern	—	1	1	1.8	—	—	—	—	—	—	—	—
No. 5	—	1(2)	1	1.8	—	—	—	—	—	—	—	—
Total	9	46	55			46	46		7	19	26	
Average grade	1.91				1.83				2.11			

(1) *Rejected, mouldy.* (2) *Rejected.*

In the group of samples collected early in the harvest season before any rain had fallen, the average grades were 1.07, 1.20, 1.00 for stook-threshed, straight-combined and swath-combined samples respectively. The lower average grade of the straight-combined samples probably has no relation to the method of harvesting in this instance, but is associated with the fact that most of them were collected from the dry area of south-central Saskatchewan. The lower average grade, therefore, was attributable mainly to low weight per bushel.

The group representing collections after the first rain showed a slight decrease in average grade of the stook-threshed, an increase from 1.2 to 1.0 in the straight-combined and a marked decrease in average grade in the swath-combined, 1.0 to 1.56. As the number in this group was small, not much significance can be attached to these changes.

The samples collected in the latter part of the harvest season showed a very distinct lowering of grade with all methods of harvesting. Compared with the early harvest samples, the grade lowering was 0.84, 0.63 and 1.11 for the stook-threshed, straight-combined and swath-combined samples respectively.

Comparison of average grades for the various groups in 1932, 1933 and for the two years collectively can be made by means of the summary given in Table VIII. The grades for the 1933 stook-threshed and straight-combined samples were lower than those of 1932, but the 1933 swath-combined samples graded higher than those of 1932. Considering both years' samples collectively, it is evident that under early season conditions, before rains occur, the swath-combine method yields the best grade and the straight-combine method the lowest grade, the average values being 0.86, 0.93 and

TABLE VIII

AVERAGE GRADES FOR COMMON WHEAT IN 1932, 1933 AND IN THE TWO YEARS COLLECTIVELY

	Average grades			
	Before rain	After one rain	After two or more rains	All samples
Stook-threshed—				
1932	0.87	0.60	1.20	1.04
1933	1.07	1.10	1.91	1.37
Collectively	0.93	1.00	1.52	1.18
Straight-combined—				
1932	0.96	0.80	1.80	1.24
1933	1.20	1.00	1.83	1.32
Collectively	1.14	0.84	1.80	1.28
Swath-combined—				
1932	0.60	0.50	2.27	1.40
1933	1.00	1.56	2.11	1.28
Collectively	0.86	1.44	2.21	1.36

1.14 for swather, stook-threshed and straight-combine methods. On the other hand, during the latter part of the harvest season, after several rains have occurred, the order is changed and stook-threshing yields the best grade and the swath-combining the poorest, the average values being 1.52, 1.80 and 2.21 for stook-threshing, straight-combining and swath-combining respectively.

Durum Wheat

In 1933 there were collected in Manitoba 172 durum wheat samples. These were considered with the common wheat in the discussions of moisture, but must be considered as a separate group for discussion of grade. A summary of the distribution in the grades and the average grades is given in

TABLE IX

SUMMARY SHOWING THE GRADES OF DURUM WHEAT SAMPLES COLLECTED IN 1933

Method of harvesting	No. of samples	% of samples grading				Average grade
		1 A.D.	2 A.D.	3 A.D.	4 A.D.	
Stook-threshed—						
Before rain	15	80.0	20.0	–	–	1.20
After one rain	6	66.6	33.4	–	–	1.33
After two rains	43	72.1	27.9	–	–	1.28
						Mean 1.29
Straight-combined—						
Before rain	15	46.7	26.7	26.7	–	1.87
After one rain	2	100	–	–	–	1.00
After two rains	13	38.5	38.5	23.0	–	1.83
						Mean 1.79
Swath-combined—						
Before rain	28	60.7	39.3	–	–	1.39
After one rain	6	83.3	16.7	–	–	1.17
After two rains	44	11.4	34.1	50.0	4.5	2.48
						Mean 2.00

Table IX. For computing average grades of durum wheat the arbitrary values 1, 2, 3 and 4 were assigned to the grades 1 A.D.*, 2 A.D., 3 A.D. and 4 A.D. respectively.

Differences in average grade between samples collected early and late in the harvesting season were small in the stook-threshed and straight-combined grain, but relatively large in the swath-combined grain. Average grades for the three methods in the above order were 1.29, 1.79 and 2.00 respectively, showing that the straight-combined samples were, on the average, one half grade and the swath-combined samples three-quarters of a grade lower than the stook-threshed samples. These results are somewhat different from those

*Amber durum.

obtained in the previous season when it was found that the stook-threshed and straight-combined samples were not appreciably different in average grade and the swath-combined samples were only lower by about one-seventh of a grade.

The average grades were lower in 1933 than in 1932 for the samples collected, as can be seen from the comparative values given in Table X.

TABLE X

SUMMARY OF THE AVERAGE GRADES OF DURUM WHEAT SAMPLES FOR 1932, 1933 AND FOR THE TWO YEARS COLLECTIVELY

Method and year	Before rain	After one rain	After two or more rains	All samples
Stook-threshed—				
1932	1.02	1.10	1.55	1.25
1933	1.20	1.33	1.28	1.29
Collectively	1.06	1.19	1.41	1.26
Straight-combined—				
1932	1.11	1.00	1.50	1.23
1933	1.87	1.00	1.83	1.79
Collectively	1.45	1.00	1.64	1.45
Swath-combined—				
1932	1.01	1.80	2.10	1.40
1933	1.39	1.17	2.48	2.00
Collectively	1.13	1.42	2.32	1.67

Relation of Harvesting Method to Moisture and Grade of Barley in 1933

In 1933 a number of barley samples were collected and although these are too few to afford reliable conclusions, they are being included as a matter of record and to give some indication of what might be expected of the three harvesting methods. Since there were only 32 samples altogether, no attempt has been made to divide them into groups according to weather, as was done in the case of wheat. It might be noted, however, that 23 of the 32 samples were collected during the period August 8-11, inclusive. The results are given in Table XI.

TABLE XI

SUMMARY OF OBSERVATIONS ON BARLEY SAMPLES COLLECTED IN 1933

Method of harvesting	No. of samples grading					Total	Average grade*	Average moisture, %
	3 Ex. C.W.	3 C.W.	4 C.W.	5 C.W.	6 C.W.			
Stook-threshed	5	5	1	–	–	11	3.6	10.7
Straight-combined	1	3	1	–	1	6	4.5	10.6
Swath-combined	4	7	1	2	1	15	4.3	10.6

**In computing average grades, the empirical values 3, 4, 5, 6, 7 were assigned to the grades 3 Ex. C.W., 3 C.W., 4 C.W., 5 C.W. and 6 C.W. respectively.*

No tough or damp samples were obtained by any of the harvesting methods. The average moistures were 10.7%, 10.6% and 10.6% for stook-threshed, straight-combined and swath-combined samples respectively, indicating no difference as a result of the methods. With respect to grades, the average values were 3.6, 4.5 and 4.3 for the three methods in the order named above. The stook-threshed samples were 0.7 to 0.9 grades higher than those obtained by the other two methods. There was little difference in grade between the straight-combined and swath-combined samples. Summing up the observations on barley it may be said that with 32 samples collected in 1933 harvesting method made no difference in moisture content and that the stook-threshed samples were, on the average, about three-quarters of a grade better than those threshed by the other two methods.

Conclusions

The 1933 study of the three methods of harvesting wheat, namely, stook-threshing, straight-combining and swath-combining, confirmed the conclusions drawn from the study of the previous year in respect to moisture content of the threshed grain. In each year it was found that 25% of all straight-combined wheat samples were tough or damp. With swath-combining there were 9% and 7% in 1932 and 1933 respectively, or 8% of the collective samples of both years. Stook-threshing showed only 3% and 10% respectively or 5.4% for the collective samples of both years. It must not be inferred that these percentages are thought to represent the whole crop in these years. Particularly in the case of the stook-threshed samples, it should be kept in mind that these were collected in the combine areas to serve as a means for comparison. They represent, therefore, only the more southerly areas where the grain tends to mature early. In either of these years, samples from the northerly areas would doubtless have shown incidence of tough and damp samples in excess of 5.4%.

In both 1932 and 1933, the harvest season in the middle and southern parts of Western Canada, where the combining method is used, was particularly favorable inasmuch as the crop ripened rapidly and quite uniformly and weed growth was not excessive. With 25% of the straight-combined wheat turning out tough and damp under such conditions, it is likely that the percentage would increase considerably in a poor harvest season. The reason for this high percentage of tough and damp wheat seems to be that the wheat straw becomes brittle enough for good threshing before the grain itself is dried to below 14.4% moisture, and the operators, fearing damage to the standing grain, become overanxious and start harvesting too early.

Reference

1. Larmour, R. K., Geddes, W. F. and Cameron, D. Can. J. Research, 9 : 486-501. 1933

A STUDY OF THE RESPIRATION AND HEATING OF DAMP WHEAT[1]

BY R. K. LARMOUR[2], J. S. CLAYTON[3] AND C. L. WRENSHALL[3]

Abstract

Respiration and heating studies were made on hard red spring wheat.

Estimation of the true respiration of hard red spring wheat is complicated by the respiration of fungi which develop on damp wheat. The germination and growth of fungi can be controlled effectively by toluene or carbon tetrachloride vapor. In the presence of vapor of these substances carbon dioxide production goes on at a low rate and no heating occurs in wheat of 25% moisture content. The odor of the vapor disappears in the course of air-drying.

Exposure of damp wheat to carbon tetrachloride for 25 days produced no deleterious effect on the quality.

The problem of storage and transportation of damp wheat assumes grave proportions in years when protracted rainfall occurs during the latter part of the harvest season. Neither farmers nor the country elevators have means for drying wheat and it must therefore be shipped with dispatch to the terminal elevators where it can be dried to a moisture content suitable for storage. When there is much damp wheat in the country, heavy losses from heating occur in storage in farmers' bins, in country elevator bins and in transit. There is no remedy for bin-burned or heated wheat; it is irreparably damaged and is fit only for feed. It cannot be blended with sound wheat even in small amounts because the moldy odor is very persistent and carries through into the flour.

In normal seasons the small amount of damp wheat that comes on the market can be easily handled either by rushing it rapidly to the driers at the terminals or by mixing it with normal dry wheat. In the latter case distribution of moisture takes place rapidly and if the mixing is done skilfully, there is little danger of heating.

With the advent of the combine harvester, a new factor was introduced, inasmuch as there seemed to be a tendency on the part of the operators to cut the grain too early. This is quite natural, as early cutting reduces the risk of damage by bleaching and loss by shelling. Larmour, Geddes and Cameron (7) in an extensive survey of harvesting methods showed that combined wheat, on the average, tends to be higher in moisture than stook-threshed wheat, but the amount of damp wheat occurring as a result of combine harvesting should present no serious handling problem in normal years. On several occasions, however, statements have been made to the

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effect that combined wheat of apparently normal moisture content tends to heat more readily than stook-threshed wheat and a number of cases of spoilage in transit have been reported. To account for this the theory has been advanced that the combined wheat, having had no chance to "sweat" before harvesting, undergoes this process in the bin or car and this provides conditions favorable for heating. In the older method of harvesting "sweating" occurs in the stook, where the small bulk and the comparatively great aeration precludes the danger of heating. It seems probable that in the course of desiccation of wheat during the latter part of the ripening process there is a stage at which there occurs a redistribution of bound and free water in the grain, accompanied by a syneretic effect which makes the grain feel damp, even though there has been no absolute change in the moisture content. Unless it is assumed that some very fundamental change in the wheat berry takes place in the course of sweating, this phenomenon alone would scarcely be sufficient to explain the observations referred to above.

The respiration and heating of cereal grains has attracted the attention of many workers on account of the practical significance of the relations of moisture content, heating and the keeping quality of the stored grain. Among the early workers Kolkwitz (6) using barley, and Quam (14) using oats, showed that the rate of carbon dioxide production increased with increasing moisture content after a certain value of the latter had been attained. Bailey and Gurjar (1), using wheat, showed that respiration depended upon the following variable factors: moisture content, temperature, concentration of carbon dioxide, oxygen supply, period of dampness, and also was partially governed by inherent factors in the wheat itself, such as protein content, plumpness and frost damage.

The role of micro-organisms in heating of stored organic material was pointed out as early as 1907 by Miehe (9), who observed their presence in heating hay. Peirce (13) and later Darsie, Elliott and Peirce (3) showed that micro-organisms had much to do with rise in temperature of stored seeds. More recently the investigations of Gilman and Barron (4), Miehe (10), Norman (11), Bakke and Noecker (2), Isatschenko *et al.* (5) and Swanson (17) have shown definitely that heating of damp grain and other organic substances such as hay and straw is usually accompanied by growth of fungi. While the heat produced in stored damp grain is doubtless due to both respiration of the embryo and the growth of fungi, several of the aforementioned workers seem to favor the suggestion that the micro-organisms are mainly responsible for the high temperatures which result in bin-burning. To date there has not been reported any very successful segregation of these two factors, the difficulty in this connection being to sterilize the grain without injuring the embryo.

In the investigation herein reported it was found possible to prevent mold growth without permanently inactivating the embryo and there was some evidence that the concentration of the inhibitors necessary to prevent incubation and growth of fungi was low enough to allow respiration of wheat.

Experimental

In this study the rate of respiration was estimated by determining the carbon dioxide production of wheat at various moisture contents isothermally and by observing the temperature changes in 15-lb. samples of damp wheat stored in insulated containers.

In the *measurement of carbon dioxide production*, two general methods were used; in one the samples of wheat were allowed to remain in closed containers for 23 hr. and the carbon dioxide was aspirated off for one hour; in the other carbon dioxide free air was drawn through the wheat continuously except for the time required to make a titration each 24 hr. With both these procedures the carbon dioxide was absorbed in standard barium hydroxide solution.

In the *measurement of temperature changes* it was necessary to use quite large quantities of wheat. After a number of preliminary trials it was found that 15-lb. samples in earthenware crocks, heavily insulated with wood-shavings, would show temperature rises at certain moisture contents. The temperatures were measured by means of long-stemmed accurate mercury thermometers inserted in the centre of the mass of wheat.

Comparison of the Continuous and Discontinuous Methods for Removal of Carbon Dioxide

In making a choice between the continuous and discontinuous methods for studying the carbon dioxide production of damp wheat, a number of facts must be considered. In the first place, a discontinuous method more closely approximates actual commercial conditions, in which the grain is stored in bins with no great chance of aeration. Furthermore this method permits the handling of a large number of samples with only one or two absorption trains. On the other hand, it has been generally recognized that accumulation of carbon dioxide in damp wheat tends to retard the respiration process and therefore the discontinuous method does not give the maximum rate of carbon dioxide production for a given moisture content and temperature. Commercially stored wheat may be subjected to "turning", both in the process of handling and for the express purpose of cooling the wheat, and thus it undergoes varying degrees of aeration. In order to estimate the difference between the two methods, determinations were made for three days on wheat at 20% moisture content. The results, given in Table I, show a very great difference in respiration rate. The continuously aerated sample not only was higher on the first day, but also increased in rate more rapidly than the non-aerated sample.

TABLE I

COMPARISON OF THE CONTINUOUS AND DISCONTINUOUS AERATION METHODS OF CARBON DIOXIDE DETERMINATION

(Wheat at 20% moisture content)

Days after tempering	Mg. of CO_2 per 100 gm. of dry wheat per 24 hr.	
	Continuous aeration	Discontinuous aeration (CO_2 swept out at end of 23 hr.)
7	59.6	21.0
8	81.4	19.6
9	131.8	39.3

TABLE II

EFFECT OF SIZE OF THE CONTAINER ON RESPIRATION RATE OF A GIVEN QUANTITY OF WHEAT

(500 gm. wheat at 20% moisture content in each case. This quantity of wheat occupied approximately 600 cc.)

Days after tempering	Mg. CO_2 per 24 hr. per 100 gm. dry wheat		
	650-cc. bottle	1000-cc. bottle	2000-cc. bottle
1	18	18	18
2	28	24	23
3	37	39	36
4	38	63	53
5	40	67	77
6	39	64	95
7	37	61	103
	Half of each sample of wheat was removed		
8	62	90	141
9	74	100	144
10	85	99	137
11	92	101	141

Furthermore, it was observed that with the discontinuous method the rate of carbon dioxide production depended to some extent on the free air space in the vessel. This is illustrated by the data in Table II, which show, as would be expected, that increasing the size of the container for a given quantity of wheat increases the amount of carbon dioxide produced. This indicates that factors such as weight per bushel and degree of packing the sample would affect the rate of carbon dioxide production in small containers.

For comparative work the continuous method seemed less subject to error and accordingly was adopted.

Acceleration of the Rate of Carbon Dioxide Production

Before proceeding to a discussion of the effect of moisture on the respiration rate, it is necessary to consider the time effect. Bailey and Gurjar (1) allowed the tempered wheat to stand for three days and then sealed the samples in containers and left them for four days, after which the carbon dioxide was aspirated off and estimated. In most of their work the rates of carbon dioxide production were calculated on the basis of this four-day period. The data in Table II show that after about three days from tempering the rate of carbon dioxide production in the two smaller containers approached a constant value which varied with the free air space in the container. In the largest bottle, which had about 1400 cc. free space above the wheat, there was an increase in rate of carbon dioxide production in each successive 24-hr. period for the first seven days. This is similar to the observations made by the continuous aeration method. It is evident, therefore, that given the proper conditions, damp wheat tends to increase in rate of carbon dioxide production; it certainly does not give a constant rate four days after tempering, except when the respiration is inhibited by the presence of carbon dioxide in too great concentration. The acceleration of rate of carbon dioxide production was not due to heating of the wheat, because the samples were too small; many measurements showed that under the conditions of these experiments the wheat did not get above room temperature, which was maintained at 22 ± 1° C. This effect, therefore, must have been due either to progressive stimulation of embryonic activity or to fungal growth. Examina-

tion disclosed the fact that samples which had "respired" at a high rate were heavily infected with fungi, mostly of the *Penicillium* type, with a few *Aspergilli*. Means were then sought for destroying the fungus spores or of inhibiting their germination and growth.

The Effect of Toluene on Carbon Dioxide Production

The work of Tomkins (18) has thrown considerable light on the action of gases and volatile substances on the growth of mold fungi. Working with acetone, acetaldehyde, hydrogen cyanide, hydrogen sulphide, sulphur dioxide and ammonia, he found that these substances either retarded the germination of the spores or inhibited their growth, or both. Swanson (17) showed that Ceresan, the active principle of which is ethyl mercuric chloride, is effective in preventing mold growth. As none of these reagents seemed to be suitable for use on milling wheat, especially on a commercial scale, it was decided to try the effect of other, more suitable substances, and the first chosen for investigation was toluene.

By the continuous aeration method, toluene vapor was introduced into the air stream by passing the air through a bubbler containing liquid toluene. The concentration of vapor thus obtained was not high; a 24-hr. run vaporized approximately 1 cc. of the liquid. In all tests of fungus inhibitors a control consisting of untreated wheat of the same lot was tested at the same time. A typical set of data obtained on toluene-treated and control samples of wheat tempered to 20% moisture content is given in Table III. In these data it should be noted that with both the treated and untreated samples, the average rate of carbon dioxide production for the fourth, fifth, sixth and seventh days after tempering was practically the same. This corresponds to the period during which Bailey and Gurjar (1) made their measurements of respiration rate. It seems likely that at 22° C., growth of the fungi does not get under way rapidly until after seven days from tempering, in wheat of 20% moisture content. If this conclusion is correct, it can be stated further that a four-day exposure to toluene vapor has no deleterious effect on the true respiration of the wheat itself, because during this period the treated and untreated samples showed the same carbon dioxide production rate. After the

TABLE III

EFFECT OF TOLUENE VAPOR ON CARBON DIOXIDE PRODUCTION OF WHEAT AT 20% MOISTURE

Days after tempering	Mg. CO_2 per 100 gm. dry wheat per 24 hr.			
	With toluene		Control—without toluene	
2	23.6		—	
3	25.4		30.4	
4	27.9	Average 29.1 (days 4–7)	27.3	Average 28.1 (days 4–7)
5	27.7		28.9	
6	28.7		26.2	
7	32.3		36.0	
8	32.8		63.8	
9	36.8		105.0	
10	32.0		133.0	
11	33.2		160.8*	
12	39.8		77.8	
13	36.5		43.9	
14	30.8		37.1	

**Toluene added after this determination was completed.*

seventh day, however, the rate of carbon dioxide production in the control sample increased very rapidly until on the eleventh day it was about 5.3 times the initial rate. During the same period, the amount produced by the toluene-treated sample increased from 32.3 to 33.2 mg. per 100 gm. per 24 hr., or from the average rate of 29 to 33, an increase that is likely within the experimental error of the method. It was concluded that the toluene vapor had either killed the spores themselves or the fungi as fast as they germinated or had simply inhibited the germination of the spores. Introduction of toluene vapor to the control sample at the end of the eleventh day resulted in a rapid diminution of carbon dioxide production and by the fourteenth day the rate on the control had dropped from 161 to 37. This was considered as evidence that toluene vapor either killed the fungi or reduced their respiratory activity to negligible proportions. It will be seen later that the second alternative is the more probable.

An attempt was made to surface sterilize the wheat, in order to see if it would be possible to get an estimate of the true respiration rate, without having to use toluene. Mead (8) in a study of various sterilizing agents found that silver nitrate and mercuric chloride solutions effected very good sterilization in respect to fungi, without seriously damaging the germination. Accordingly a sample of wheat was immersed in 0.1% mercuric chloride solution for 10 min., after which the grain was thoroughly washed. Carbon dioxide production of the samples with and without toluene was measured. The results are given in Table IV.

TABLE IV

Carbon dioxide production of wheat of 25% moisture content, previously immersed for 10 min. in 0.1% mercuric chloride solution and then washed

Days after tempering	Mg. CO_2 per 100 gm. dry wheat per 24 hr.	
	With toluene	Control—without toluene
4	120	82
5	125	86
6	122	86
7	97	77
8	76	71
9	58	70
10	41	71
11	28	74
12	24	87
13	25	119
14	16	133
15	11	167
16	9	181
17	7	222

Examination of the samples after the run had been made showed the following:

Fungus infection before incubation	0%	31%
Infection after incubation	93% (mostly bacteria)	98% (mostly *Penicillium*)
Germination	0%	0.5%

The samples treated with toluene vapor commenced producing carbon dioxide at a higher rate than the control samples, but as time went on the rate decreased, until finally on the seventeenth day after tempering, the rate was down to 7. The control samples showed a tendency to decrease in rate

until the thirteenth day, when the trend reversed and a rapid increase occurred during the last five days of the experiment. The initial stimulation which was apparently due to the toluene vapor is analogous to results obtained by Passerini (12), who observed that a short immersion of seeds in carbon disulphide or carbon tetrachloride accelerated germination. While slight increases in carbon dioxide production, attributable to toluene or carbon tetrachloride vapor, have been observed in many instances, at no time has the effect been as great as in this particular case. It can only be assumed that the immersion in the mercuric chloride solution made the embryo more sensitive to stimulation.

After the respiration "run" was finished, the samples of wheat were submitted to Mr. Mead for examination; his observations are given at the bottom of Table IV. The wheat had entirely lost its viability; the sample treated with toluene vapor showed no fungus infection, but after incubation 93% of the kernels were found to be infected with bacteria; the control sample was 31% infected with fungal growth and after incubation 98% of the kernels examined were infected, mostly with *Penicillium*. It is obvious from these data that the treatment with mercuric chloride solution was ineffective as a sterilizer for fungi. Furthermore, it weakened the viability of the embryo to such an extent that exposure to toluene vapor on the one hand, and growth of fungi on the other, destroyed the germination entirely. It would therefore be out of the question to attempt to measure the true respiration rate by this method.

In order to ascertain the effect of exposure to toluene vapor on the viability of wheat the following experiment was made. Samples of the one lot of wheat were tempered to moisture contents of 10.5, 12.5, 15, 17.5, 20.0, 22.5 and 25%. These were placed in sealers at the bottoms of which were open beakers protected by wire gauze, containing toluene, and left for four days, after which time they were exposed and spread out in the laboratory for two days. Germination tests made on these samples gave the results shown in Table V.

It is evident that exposure to toluene vapor tends to inactivate the embryo permanently and, therefore, while it is effective in preventing growth of molds, it could not be applied in this study because of the difficulty of estimating how much of the retardation of carbon dioxide production was due to inhibition of molds and how much to inactivation of the embryo. The fact that it lowers the viability seriously and furthermore that it is inflammable renders toluene inapplicable commercially.

TABLE V

GERMINATION TESTS ON WHEAT OF VARIOUS MOISTURE CONTENTS PREVIOUSLY EXPOSED TO TOLUENE VAPOR FOR FOUR DAYS

Moisture, %	Germination, %
25	16
22.5	15
20	35
17.5	33
15	60
12.5	70
10.5	64
Control sample	92

In some tests made with a non-inflammable mixture of toluene and carbon tetrachloride it was found that the mixture prevented mold growth as effectively as the pure toluene. This led further to the observation that pure carbon tetrachloride was an efficient fungus growth inhibitor. Thereafter attention was directed solely to the investigation of the behavior of carbon tetrachloride on damp wheat, because this substance, if effective, possesses characteristics making it ideally suited to commercial application; it is low in price, is non-inflammable, being in fact used extensively as a fire extinguisher, and is reputed to be a fairly good insect repellent. If it could be applied in effective concentration without damaging the wheat for storage or for milling and baking purposes, it might be used commercially to prevent damage to damp wheat in transit to the terminal elevators.

The Effect of Carbon Tetrachloride on Carbon Dioxide Production of Damp Wheat

Carbon dioxide production measurements were made on samples of wheat tempered to 12, 14, 16, 18, 20, 22 and 24% respectively. The runs were started in each case three days after tempering. The carbon tetrachloride vapor was introduced by means of a bubbler containing water and carbon tetrachloride. Runs were continued for eight days, after which the wheat was examined carefully for signs of mold and was given a germination test. The observations are presented in Table VI.

The carbon tetrachloride vapor prevented mold growth in all except the 24% moisture sample. No molding of the control samples occurred at 16% or lower moisture, but at 18% and higher moistures the wheat molded and the germination was impaired. The samples exposed to carbon tetrachloride vapor showed no evidence of reduction of viability except in the case of the 24% sample. This sample, which was at a higher moisture than is ordinarily found in commercial samples of damp wheat, probably should not have been allowed to stand for three days after tempering, because at this high moisture content germination of spores might have started.

Attention should be directed to the progressive decrease in carbon dioxide production rate of the higher moisture samples treated with carbon tetrachloride vapor. At first this looked like a decrease in embryonic activity resulting from prolonged exposure to the vapor, but the germination data belie the suggestion of damage to the embryo. Another explanation of this decrease in rate might be sought in the decrease in moisture content of the samples during the course of the experiment. This change in moisture content is a serious difficulty with the continuous aeration method and can be overcome only by using solutions the vapor pressure of which is in equilibrium with the wheat sample under examination. At the time these experiments were being conducted there was not sufficient information available on this subject, and consequently the data herein presented must be discounted somewhat. However, the drop in moisture content is not adequate as an explanation of the decrease in rate of carbon dioxide production noted

TABLE VI

EFFECT OF CARBON TETRACHLORIDE VAPOR ON THE RATE OF CARBON DIOXIDE PRODUCTION AND ON GERMINATION OF WHEAT AT VARIOUS MOISTURE CONTENTS

Initial moisture content	Mg. CO_2 per 24 hr. per *100 gm. wheat*													
	12%		14%		16%		18%		20%		22%		24%	
Days after tempering	Control	CCl_4	Control	CCl_4	Control	CCl_4	Control	CCl_4	Control	CCl_4	Control	CCl_4	Control	CCl_4
4	4.5	3.2	4.5	2.6	6.7	4.4	17.5	5.5	18.4	19.4	48	37	74	100
5	3.9	2.6	4.0	2.6	4.0	3.4	21.3	6.2	18.4	16.3	51	34	87	111
6	3.9	2.6	3.3	2.6	4.7	3.4	33.2	5.5	19.8	14.1	48	31	95	98
7	3.2	3.2	5.2	2.6	5.1	3.4	41.4	4.8	28.3	12.4	47	29	104	87
8	2.6	2.0	3.3	2.6	5.1	3.4	43.5	5.5	37.4	10.6	52	28	113	80
9	4.5	3.2	4.0	3.3	6.7	4.0	44.2	4.2	42.3	7.8	64	27	120	73
10					5.4	4.0	44.2	3.5	48.1	8.4	81	26	136	75
11					4.0	3.4	47.0	3.5	50.9	8.1	96	28	149	75
Moisture at end of run	12.3	12.0	13.8	13.3	15.6	15.7	19.5	17.5	19.9	19.1	22.3	21.4	23.8	23.9
Germination after the run	92	100			96	92	76	92	68	98	32	92	32	62
Condition after the run	normal	normal	normal	normal	normal	normal	moldy odor	normal	quite moldy	normal	very moldy	normal	very moldy	trace moldy

Table VI, because in the case of the 24% moisture sample, in which the ecrease of rate was greatest, there was an insignificant decrease in moisture ontent of the wheat.

There are two other possible explanations, one being that the first effect f carbon tetrachloride vapor is a stimulation of the embryo, the other being hat there is a progressive inhibition of respiratory activity, an anaesthesia effect, which slows down the metabolism without injuring the organism. This effect is noticeable only in the samples of higher moisture content. The anaesthesia explanation gets some support from the fact that wheat will not germinate in an atmosphere even of relatively low carbon tetrachloride vapor concentration, but on change from that atmosphere to one free from the vapor will germinate 67%. Results of such an experiment are given in Table VII.

TABLE VII

EFFECT OF CARBON TETRACHLORIDE VAPOR ON GERMINATION OF WHEAT

Treatment	Germination, %
1. Control	100
2. 0.1 cc. CCl_4 in 10 litre vessel	92
3. 1.0 cc. CCl_4 in 10 litre vessel	0
4. Sample 3 in 10 litre vessel free from CCl_4 vapor	67

In each case the germination tests were made in a 10 litre desiccator with closely fitting top. For No. 4, the sample was removed and blown free of carbon tetrachloride vapor and the vessel was carefully swept free of the vapor; the sample was then returned and tested in the usual way. The lowered viability may have been due to actual damage to the embryo or to residual carbon tetrachloride that had become absorbed by the wet wheat. It will be shown later that damp wheat does absorb the vapor to considerable extent. The important fact in these data is that, although the wheat was still viable, it gave not the least sign of germination in an atmosphere in which the concentration of carbon tetrachloride vapor could not have been greater than 18 cc. per 1000 cc., provided all the carbon tetrachloride evaporated at once and none of the vapor was removed by absorption or hydrolysis. It seems quite probable, therefore, that the decreases noted in Table VI may be accounted for, in part at least, by assuming that carbon tetrachloride vapor tends to inhibit embryonic activity.

The foregoing discussion points to the conclusion that a relatively low concentration of carbon tetrachloride vapor constantly maintained is quite effective in preventing the usual rapid increase in carbon dioxide production associated with germination and growth of fungi in wheat of 18% or higher moisture content, and that such effective concentrations of carbon tetrachloride vapor have little or no deleterious effect on the viability of the wheat. In considering the moisture levels in Table VI, it should be kept in mind that these determinations were conducted under isothermal conditions, not at all comparable to those to be found in large bulks of grain. Therefore, no conclusions can be drawn from these data as to the safe moisture limits of

untreated commercial damp wheat. It can be said, however, that wheat of 18% moisture content can be stored without danger of damage by heating if treated with an adequate concentration of carbon tetrachloride vapor, because under these conditions the carbon dioxide production rate is of the same order as that of 12% wheat. It will be shown later, in the discussion of "heating of damp wheat", that wheat at 25% moisture content can be prevented from heating by proper use of carbon tetrachloride, and on this basis, it may be stated tentatively that the carbon dioxide production rates shown in Table VI for samples at 22 and 24% moisture treated with carbon tetrachloride are likely too low to start heating, but there is no direct evidence for this assumption.

The Effect of Moisture on Carbon Dioxide Production of Wheat

The term "carbon dioxide production" has been used in place of "respiration" because it is evident that there are two kinds of respiration in damp wheat, one due to the slow metabolism of the semi-dormant embryo of the wheat, the other due to the active metabolism of the rapidly growing fungi. From the standpoint of the commercial problem of heating it is important to separate these two respirations, especially if it is found possible to control the activity of fungi, because it would be necessary to ascertain whether the respiration of the wheat alone could produce enough heat to affect the condition of the grain seriously. If we consider the problem as it affects the present methods of handling damp wheat, it probably is not necessary to differentiate these respirations, because they both work to the same end, namely, an increase in temperature of the mass of wheat, accompanied by acceleration of respiration of both the wheat and the fungi until a maximum temperature is reached, by which time the grain has become definitely spoiled. Whichever way the problem is regarded, it will be very difficult to come to a definite conclusion.

From the data in Table VI, it is evident that there is no tendency toward acceleration of rate in either the treated or control samples at or below a moisture content of 16%. At some moisture value between 16 and 20% the control samples showed a marked acceleration of rate. Unfortunately the control at 18% underwent an unaccountable increase in moisture content during the course of the run, getting up to 19.5% at the finish, and the values for that particular sample are unreliable. However, judging from the rates shown by the treated sample, it would seem reasonable to suppose that the rapid acceleration starts at moistures closer to 20% than to 16%. It is not important to establish this point definitely for laboratory conditions because, as mentioned before, these observations were made on small samples of grain under approximately isothermal conditions. Furthermore, the moisture point at which a rapid acceleration starts must vary with other factors such as temperature, kind, and soundness of grain.

It is important to know that beyond some definite critical moisture limit the rate of total carbon dioxide production increases very sharply and acceler-

ates with time even under isothermal conditions. This acceleration is doubtless due to multiplication of fungi. The differences between the carbon dioxide production rate on the fourth and eleventh days give some measure of this effect, but the difference cannot be attributed wholly to the respiration of the fungi, because we cannot say definitely that the rate on the fourth day after tempering represents only wheat respiration. It may be stated emphatically that the carbon dioxide measurements on wheat of 22% moisture or lower, taken on the fourth and fifth days after tempering, give as good an estimate of wheat respiration at 22° C. as can be obtained.

The rates given by the treated samples are probably lower than the true respiration rate for wheat at these moistures, because, as pointed out previously, there is evidence for believing that carbon tetrachloride vapor tends to inhibit embryo activity in the wheat, especially at the higher moistures. The differences between the carbon dioxide production rates of the treated and check samples on the eleventh day represent the controllable part of the total carbon dioxide production of damp wheat. It should be particularly noted that the action of carbon tetrachloride vapor raises the critical moisture limit by at least 2%, in this case definitely from 16% to 18 or probably 20%. This means that wheat at 18% moisture content respires no faster in the presence of carbon tetrachloride vapor than wheat at 12% moisture and therefore *could not heat* under these conditions. Even at 20% the initial high rate of 19 is reduced in nine days to a rate of 8 which would seem to be low enough to preclude the possibility of heating. Indeed it seems extremely probable that the highest final rate recorded for the treated samples, namely 75, is not sufficient to cause heating if fungal growth can be prevented. Evidence for this is to be found in the discussion of heating which follows.

The Heating of Damp Wheat

Since reference to the methods used to study heating of wheat has been made in the first part of the paper, consideration of the results most pertinent to this subject may now be undertaken without discussion of the many preliminary experiments.

The Effect of Moisture on Heating

In order to get some information regarding the time required to reach maximum temperature, four 15-lb. samples were made up to 16.1, 18.1, 19.5 and 21.5% moisture content respectively and kept under observation for 19 days. The data obtained are given in Table VIII.

At 16.1% moisture the temperature of the wheat did not rise more than 2° C. above room temperature; at 18.1% moisture the temperature started rising slowly at the seventh day and by the nineteenth day had risen to about 31° C., eight degrees above room temperature; at 19.5% moisture a maximum of 44.7° C. was recorded on the sixteenth day; at 21.5% moisture a maximum of 44.5° C. was recorded on the ninth day.

TABLE VIII

TEMPERATURE OF WHEAT STORED AT VARIOUS MOISTURE CONTENTS

Days	Temperature of sample in °C.			
	1	2	3	4
0	23.7	23.2	24.0	23.8
2	20.0	20.5	21.3	22.0
4	20.2	21.1	21.4	26.0
5	20.5	22.0	22.7	29.0
7	21.2	23.0	28.2	36.0
9	22.0	24.5	35.5	44.5
10	22.2	26.0	39.0	44.0
11	22.5	26.0	39.0	43.0
12	22.5	27.0	40.0	43.0
16	24.0	31.0	44.7	44.0
17	24.2	30.5	44.0	42.7
18	24.4	30.7	43.3	41.6
19	24.2	30.3	42.6	41.3
Moisture content	16.1%	18.1%	19.5%	21.5%
Final condition	sound in appearance, slight yeasty odor	pronounced musty odor	both severely bin-burned and completely spoiled	

While a difference of seven days in time was required to reach the maximum temperature of approximately 45° C. with the samples of 19.5% and 21.5% moisture content, there was only a difference of 2-3 days in time required for initiation of heating. The rate of heating, therefore, appears to be directly related to the moisture content of the wheat. It should be pointed out, too, that there was a distinct maximum of about 45° C.; after this was attained there was a tendency to decrease in temperature. This may be attributable to destruction of the embryo activity of the wheat or to killing of the fungi, or to both. It agrees with the data of Smith and Bartz (15), which show that cracked corn and crushed oats, when stored in piled sacks, attained maximum temperatures varying from 42° to 49° C., after which there was a slow decrease.

The condition of the samples at the end of the experiment is briefly described in Table VIII. Those at 19.5 and 21.5% moisture were typically bin-burned and were considered completely ruined, even for feed. The sample at 18.1% which had reached a maximum of only 31° C. had a musty odor sufficiently pronounced to degrade it to "Feed". These three samples were heavily infected with spores. In the light of the "respiration" results it appeared probable that fungus growth was responsible to a large extent for the increased carbon dioxide production which led to the increased temperatures, but since ordinary wheat respiration might produce the same temperature effect under the semi-adiabatic conditions of these experiments no definite conclusion on this point could be reached from consideration of these particular data. It was definitely shown in another experiment that initial inoculation of a 15-lb sample with 100 gm. of previously heated wheat had the effect of greatly hastening the attainment of the maximum tem-

perature. Furthermore, as will be shown by the results of the following experiment, damp wheat can be treated with carbon tetrachloride so as to prevent heating and spoilage. These considerations lead to the conclusion that growth of mold is mainly responsible for the heating of damp wheat.

Prevention of Heating of Damp Wheat

In order to reduce time and to compensate to some extent for the small bulk of the samples, the wheat used in the following experiment was tempered to 25% moisture content, a value seldom reached in commercial lots of damp wheat. The carbon tetrachloride was applied by means of a small flask containing 75 cc. of the liquid, into which dipped a loose wick of cotton wool. The top of the flask was guarded by a copper gauze hood. This crude evaporator was placed in the bottom of the 3-gallon crock and covered with 15 lb. of the wheat. Observations were made for 13 days and then the samples were unpacked and examined. All the carbon tetrachloride had evaporated and these treated samples showed very faint signs of mold. The evaporators were replenished, the wheat put back and observations continued for 10 days more. The temperatures recorded are given in Table IX.

TABLE IX

EFFECT OF VARIOUS TREATMENTS ON THE TEMPERATURE OF DAMP WHEAT; 15-LB. SAMPLES OF WHEAT AT 25% MOISTURE CONTENT WERE USED

Days after tempering	1 Control	2 CCl_4 vapor present	3 CCl_4 vapor present	4 In sealed container	Room temperature
1	25.6	25.6	25.2	27.5	21.8
2	24.9	23.3	23.5	26.3	21.7
3	24.8	22.8	23.0	26.0	21.8
4	25.6	22.6	22.8	26.1	22.0
5	27.9	22.4	22.8	26.1	22.0
6	32.7	22.3	22.8	26.0	21.6
7	39.5	22.1	23.0	25.3	21.0
8	43.9	21.8	21.8	25.0	20.6
9	45.8	21.6	21.5	25.0	21.0
10	45.9	21.6	21.5	25.2	21.5
11	45.2	21.9	22.0	25.5	22.2
12	44.2	22.1	22.3	25.5	21.7
13	—	22.0	22.2	—	21.5
17	—	22.7	22.5	25.1	
19	—	22.4	22.5	24.3	
21	—	21.6	21.5	23.8	
23	—	21.1	21.1		
Final condition	completely spoiled	*normal*	*normal*	*musty*	

The control sample reached a maximum temperature of approximately 46° C. in nine days, but the samples exposed to the carbon tetrachloride vapor showed absolutely no tendency to increase in temperature. They were about 3.5° C. above room temperature on the first day, on account of the heat of wetting, but this initial temperature had decreased to room tem-

perature by the fifth day and thereafter there was no significant increase. The final examination of these treated samples showed them to be normal in appearance, with no musty odor.

Along with these samples, there was carried one untreated sample sealed in an airtight container filled to its capacity with 20 lb. of wheat at 25% moisture. This sample showed no tendency to heat, but rather a slight tendency to drop in temperature. During the greater part of the 23-day period it was about 4° C. above room temperature. On final examination the sample was found to have a musty odor. It is evident that in sealed containers the carbon dioxide production is sufficiently retarded to prevent any marked rise in temperature, but under these conditions enough mold growth may occur to put the grain out of condition.

While the foregoing experiment furnished convincing evidence that heating of damp wheat can be prevented by carbon tetrachloride vapor, it was recognized that the production and maintenance of an effective concentration of the vapor in lots of wheat of commercial size would present a number of difficulties. Initial spraying of the wheat with carbon tetrachloride by means of an atomizer was found to be ineffective; it seems necessary to maintain continuously a certain concentration of the vapor. This might be expected on account of the fact that carbon tetrachloride does not kill the spores, but only inhibits their development. It is necessary, therefore, to use some sort of evaporator which can be depended on to supply the vapor constantly. The question then arises, how far will the vapor penetrate in damp wheat; in other words, what would be the effective range of an evaporator? On account of the great relative density of carbon tetrachloride vapor one would expect that downward displacement might be a little more rapid than upward displacement. However, Strand (16) showed that adsorption of the vapor by the top layers of grain prevents its rapid downward movement and consequently, when the vapor is applied at the top of a column of grain, the concentration of the gas varies inversely with the depth below the surface. In the aforementioned heating trials, the top of the evaporator was not more than 10 in. below the surface of the grain and the container was loosely covered with shavings to lessen air movement. Under these conditions, there evidently was maintained a concentration of carbon tetrachloride vapor sufficient to prevent mold growth, which indicated that the vapor was not adsorbed too rapidly for practical purposes. In order to get more information concerning the effective penetration of carbon tetrachloride vapor in damp wheat, the following trials were made.

Cylindrical towers were constructed of heavy waxed paper cartons 8 in. high and 6 in. in diameter. The bottom of each carton was cut away and replaced by copper gauze and they were joined by means of cardboard bands in such a way that the whole tower could be put up and taken down in segments. In setting up a run each segment was filled to capacity, so that the bottom of the upper one rested on the top of the wheat in the one below. This was as close an approach to a continuous column of wheat as could be

arranged with the equipment at hand. Of course, after charging, the wheat in each segment tended to settle, with the result that finally there were always small air spaces between the segments. Doubtless, too, there was opportunity for leakages of gas around the joints. However, it was thought that loss of vapor might compensate to some extent for lack of continuity of the mass, and at any rate the results would give some indication of the extent of effective penetration of the carbon tetrachloride vapor. Experiments were conducted with the source of carbon tetrachloride both at the bottom and at the top of the tower. Some of the results are presented in Tables X and XI.

TABLE X

Upward penetration of carbon tetrachloride vapor in damp wheat. Observations made on wheat at 19% initial moisture, in vertical segmented towers, 6 in. in diameter, each segment 8 in. high. Segments numbered consecutively from the bottom up. 200 cc. carbon tetrachloride in wide beaker placed at bottom

(Duration of experiment—10 days)

Segment	Appearance of wheat	Odor of CCl_4	Odor of mold	Final moisture content
1	sound	marked	nil	17.5
2	sound	detectable	nil	17.8
3	sound	*doubtful*	*faint*	17.8
4	some mycelia	nil	marked	17.4
5	some mycelia	nil	marked	17.4
6–13	moldy	nil	marked	17.4–15.5

During the ten days 103 cc. of CCl_4 evaporated. The fungal growth was most pronounced at the top of each segment.

TABLE XI

Downward penetration of carbon tetrachloride vapor in damp wheat. Experiment conducted as described in Table X except that 100 cc. carbon tetrachloride was poured into the top of the column and a beaker with 225 cc. CCl_4 was placed in the top segment.

Wheat at 20% moisture content

(Duration of experiment—7 days)

Segment	Appearance of wheat	Odor of CCl_4	Odor of mold
12—blank			
11	sound	strong	nil
10	sound	strong	nil
9	sound	strong	nil
8	sound	strong	nil
7	sound	fair	very slight
6	sound	slight	very slight
5	sound	slight	very slight
4	sound	doubtful	slightly stronger than 5
3	sound	slight	nil
2	sound	slight	very slight
1—blank			

By the second day the odor of CCl_4 was very distinct at the bottom and throughout the column. The CCl_4 evaporated at the rate of 20 cc. per day. There were no visible signs of mold, and on drying this wheat appeared sound.

The two sets of observations cannot be compared, because in one case, the downward diffusion experiment, the wheat was initially treated by pouring 100 cc. of liquid carbon tetrachloride into the top of the tower. It is evident, however, from Table X that the effective upward penetration of carbon tetrachloride vapor under these conditions was not more than 24 in. On the other hand, by the method described in

Table XI it was possible to maintain a fairly effective concentration of the vapor through a distance of 72 in. of wheat.

This experiment is scarcely comparable to commercial conditions, because the bottom segment was above the floor and had a small door. This might tend to facilitate the downward movement of the vapor. The trial does show, however, that enough carbon tetrachloride evaporates from a small surface to create and maintain a concentration of vapor effective in preventing mold growth even in wheat of 25% moisture content.

As a result of these and other similar observations, it is concluded that the application of carbon tetrachloride can be made most effectively by first adding the liquid, either by pouring or spraying it over the wheat *in situ* or as it is being loaded, and then maintaining a source of carbon tetrachloride at the top of the mass of wheat. It is recognized that these investigations are at best only approximations to commercial conditions and that extensive tests on large bulks of damp wheat must be conducted before a definite recommendation regarding a practical method can be made.

The Effect of Carbon Tetrachloride on the Milling and Baking Quality of Wheat

In considering the possibility of applying this treatment commercially it is essential to know how such treatment might affect the quality of flour. Accordingly a careful study was made of flour milled from wheat treated with varying degrees of severity with carbon tetrachloride.

Five 5-lb. samples of sound wheat were tempered to 24% moisture content, placed in securely stoppered bottles, to which were added dosages of 1, 2, 4, 8 and 12 cc. carbon tetrachloride respectively. They were left for 25 days and then dried and milled in the usual way. With this series there were included three control samples, one of the untreated original wheat at 12% moisture, one of the same wheat treated for 25 days with 4 cc. of carbon tetrachloride and one of untreated wheat of 24% moisture content. The miller's observations are given in Table XII.

TABLE XII

OBSERVATIONS ON THE ODOR OF FLOUR AND TASTE OF BREAD PREPARED FROM WHEAT TREATED WITH CARBON TETRACHLORIDE

Description of treatment of sample	Odor of flour	Taste of bread
1. Control (1)—original wheat at 12% moisture	normal	normal
2. Control (2)—original wheat at 12% moisture with 4 cc. CCl_4	normal	normal
3. Control (3)—24% moisture	slightly musty	normal
4. 24% moisture with 1 cc. of CCl_4	fruity odor	normal
5. 24% moisture with 2 cc. of CCl_4	fruity odor	normal
6. 24% moisture with 4 cc. of CCl_4	fruity odor	normal
7. 24% moisture with 8 cc. of CCl_4	odor of CCl_4	normal
8. 24% moisture with 12 cc. of CCl_4	odor of CCl_4	normal

After being aged for three weeks, the flours were baked by six different formulas which are described in Table XIII.

TABLE XIII

EFFECT OF TREATMENT WITH CARBON TETRACHLORIDE ON BAKING QUALITY

Description of sample	Absorption, %	Loaf volume, cc.	Texture	Crumb color	Crust color	Shape
	Simple formula					
1. Control (1)—original wheat at 12% moisture	62	725	7.5	7.5	5	5
2. Control (2)—original wheat at 12% moisture with 4 cc. CCl_4	62	650	7.5	7.5	3	4
3. Control (3)—24% moisture	62	695	6.5	7	4	4
4. 24% moisture with 1 cc. CCl_4	61	760	7.5	7.5	4	5
5. 24% moisture with 2 cc. CCl_4	60	735	7.5	8	5	5
6. 24% moisture with 4 cc. CCl_4	60	730	7	8	5	5
7. 24% moisture with 8 cc. CCl_4	61	715	7.5	7.5	4–d**	5
8. 24% moisture with 12 cc. CCl_4	61	743	7	8	4–d	5
	0.001% $KBrO_3$					
1. Control (1)—as above	62	768	7.5–o*	8	4–d	5
2. Control (2)—as above	62	808	7–o	8	4–d	5
3. Control (3)—as above	62	835	7–o	7.5	4–d	5
4. As above	61	900	6.5–o	8	4–d	5
5. As above	60	838	7–o	8	4–d	5
6. As above	60	830	7–o	8	4–d	5
7. As above	61	798	7–o	8	4–d	5
8. As above	61	866	7–o	9	4–d	5
	0.002% $KBrO_3$					
1. Control (1)—as above	62	875	5–o	8	4–d	5
2. Control (2)—as above	62	878	5–o	8	4–d	5
3. Control (3)—as above	62	773	5–o	8.5	4–d	5
4. As above	61	783	7–o	8.5	4	5
5. As above	60	783	7.5–o	8.5	5	5
6. As above	60	783	7.5–o	8.5	4–d	5
7. As above	61	770	7–o	9	4–d	5
8. As above	61	750	6.5–o	8.5	5	5
	0.003% $KBrO_3$					
1. Control (1)—as above	62	705	7.5	8	5	4
2. Control (2)—as above	62	780	8–o	9	5	4.5
3. Control (3)—as above	62	760	7	8	5	5
4. As above	61	690	7.5	8	5	4.5
5. As above	60	715	7.5	8	5	5
6. As above	60	695	7.5	9	5	4.5
7. As above	61	685	7.5	9	5	5 torn
8. As above	61	670	7.5	9	5	3.5 torn
	0.004% $KBrO_3$					
1. Control (1)—as above	62	770	7	7	5	3 torn
2. Control (2)—as above	62	750	8–o	8	4	5
3. Control (3)—as above	62	630	7	7	4	3
4. As above	61	633	8–o	8	4	3
5. As above	60	619	7	8	4	3
6. As above	60	645	7.5	8	4	4
7. As above	61	618	8	8	4	3
8. As above	61	590	8.5	7	4	3
	Malt-bromate-phosphate					
1. Control (1)—as above	62	1090	5	8	4–d	4
2. Control (2)—as above	62	1010	5–o	9	3–d	4
3. Control (3)—as above	62	830	7	8	5	5
4. As above	61	805	6.5	8	5	4
5. As above	60	865	7–o	8	5	4
6. As above	60	800	7	9	5	5
7. As above	61	750	7	8	4–d	5
8. As above	61	920	7	8	4–d	4

*o = open. **d = dark.

With the basic formula no deleterious effect of the carbon tetrachloride treatment was observed; on the contrary, there was a very slight evidence of improvement.

Four straight bromate formulas with 1, 2, 3 and 4 mg. potassium bromate respectively were used. With the lower dosage, the effect of carbon tetrachloride treatment was, if anything, favorable. With increasing dosages of potassium bromate, however, the samples that had been tempered to 24% moisture content commenced falling below the 12% moisture samples. However, comparison with the 24% moisture control sample failed to show any specific effect attributable to the carbon tetrachloride treatment.

With the malt-bromate-phosphate formula, again, all those samples initially tempered to 24% gave volumes about 200 cc. lower than the dry control samples, but comparison with the wet control sample failed to reveal differences that could be attributed to carbon tetrachloride treatment.

From these data it is concluded that prior treatment of damp wheat with carbon tetrachloride for a period of 25 days does not have any effect on the baking quality of flour milled therefrom. It was observed, however, that storage at 24% moisture content lowered the tolerance toward severe treatment with improvers.

In view of Swanson's results (17), published after this work was completed, longer exposure to the carbon tetrachloride should have been made, in order to ascertain whether or not its action is similar to that of Ceresan. Trials involving long exposures are in progress and the results will be reported in a later paper.

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BLOOD PARASITES OF RUFFED GROUSE (*BONASA UMBELLUS*) AND SPRUCE GROUSE (*CANACHITES CANADENSIS*), WITH DESCRIPTION OF *LEUCOCYTOZOON BONASAE* N. SP.[1]

By C. H. D. Clarke[2]

Abstract

A list of blood parasites found in ruffed and spruce grouse, including *Leucocytozoon bonasae* n. sp., *Trypanosoma gallinarum* Bruce *et al.*, 1911, and Microfilariae, is given. Members of the genus *Leucocytozoon* being known to be pathogenic, the possibility of a connection between *Leucocytozoon bonasae* and the problem of grouse periodicity is suggested.

For the past three years the writer has been engaged in an investigation of the cycle in numbers of grouse, particularly of the ruffed grouse, (*Bonasa umbellus* (L.)), in Ontario. The study of grouse haematozoa has been part of this, and it is felt that the time has come to record the species found.

Leucocytozoon

This parasite was first found at Frank's Bay, Lake Nipissing, Ontario, in May, 1933. Later in 1933 it was discovered at Biggar Lake, Algonquin Park, where young grouse had obviously been decimated. It was especially abundant in a smear from a very sick bird. Attention has already been called (2) to its occurrence at Brule Lake, Algonquin Park, in the summer of 1934, and its association with a heavy mortality of young birds. A similar condition was found at Frank's Bay in the same season. It is not the purpose here to discuss the possible significance of its occurrence beyond recalling that the pathogenicity of certain members of the genus is well established (5, 12, 15, 19).

Description of gametocytes. In the classification of Marcel and André Leger (6) the species under consideration is of the fusiform type, with the host-cell nucleus compact. Micro- or male gametocytes are noticeably less frequent than macro- or female gametocytes and are characterized by taking a much lighter stain, a feature applying to their host cells as well. Their size ranges overlap completely but the microgametocytes appear more commonly in the smaller limits. The cytoplasm of the macrogametocyte is dark blue (Giemsa stain) granular to alveolar, and vacuolated; that of the microgametocyte is pale blue and more uniform. The nucleus of the macrogametocyte stains a pale red, is usually more or less round, in size about 2.5 or 3μ and located anywhere within the parasite; that of the male pale red, oval, centrally located (usually), and 12 $\times$ 5μ in size. Pigment granules are found in both male and female in greatly varying amounts; female cells contain on the average more pigment. The parasites themselves measure 18 to 20μ by 6 to 7μ; often more rounded forms around 12 $\times$ 15μ are found. The host cell is

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10 to 12μ in width and varies in length from 25 to 40μ depending on the attenuate ends. Its nucleus, a dark red body, lies to one side. Its cytoplasm is seen only at the ends. These are characteristically coarsely granular in this species, with red staining chromatoid granules.

No chromatin has been found in the male nucleus, that might be interpreted as a karyosome. In the female nucleus a more or less compact group of red staining granules is often seen. Likewise no scattered chromatin granules have been found. Red staining granules have been noticed but these appear to be identical with the granules in the ends and have not been seen in parasites free from their host cell. Certain gametocytes have been found in which the host-cell nucleus lay either beneath or on top of the parasite. These forms are more attenuated (5 or 6μ × 20 to 25μ, parasite; and 35 to 55μ, host cell), and are considered immature, since they are not present to any extent in mature birds, especially in spring. Developing forms are rare in peripheral blood (9).

In size and general morphology this species most closely resembles *L. lovati* Seligman and Sambon (14) of the red grouse (*Lagopus scoticus*) and *L. mansoni* Sambon (13) of the capercailzie, (*Tetrao urogallus*). It differs in its oval shape and in the reduced length of the fusiform ends of the host cell. References to other species are appended. Obviously the two species above are closely related and the differences may be those of host only. With the limitations due to our absolute lack of knowledge of relationships within this genus, the name *Leucocytozoon bonasae* sp. nov. is proposed for the species under consideration.

Schizogony in *Bonasa umbellus*

Ruffed grouse No. 126, (in the specimens collected during the study of grouse periodicity), adult male, Frank's Bay, May 19, 1933. Also a series from Biggar Lake, July 1933, Frank's Bay, June 1934, and Brule Lake, June, July and August 1934.

Canachites canadensis

A species of *Leucocytozoon,* not sufficiently well preserved for specific determination was found in spruce grouse No. 124, adult female, Frank's Bay, May 15, 1933.

The specimens described and figures are from ruffed grouse No. 185, juvenile male, Brule Lake, August 16, 1934.

Sporogony: unknown.

Organs for the study of schizogony were preserved and are to be studied shortly.

Trypanosomes

Trypanosomes were recorded from ruffed grouse in Michigan by Stafseth and Kotlan (16) in 1925, without further identification or description. In the present study they have been found in small numbers in blood smears from

three grouse. No cultures were made owing to the limitations of a field laboratory. All specimens observed were in the large, S-shaped stage described by Novy and MacNeal (11) as the most mature.

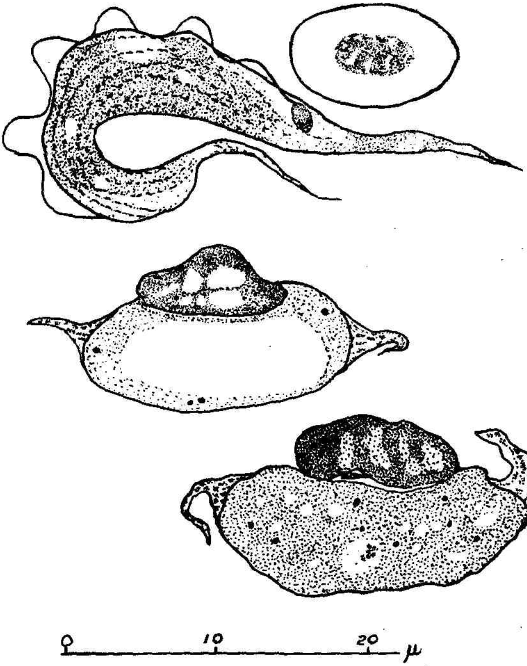

FIG. 1. *Top—Trypanosome of the ruffed grouse. Middle—Leucocytozoon bonasae, microgametocyte. Bottom—Leucocytozoon bonasae, macrogametocyte. Erythrocyte shown for comparison.*

The specimens, which are all curved as they lie on the slide, are about 55μ long by 5μ wide. The ***kinetoplast*** stains a dense red (Giemsa), is oval, 2μ × 1μ, sometimes smaller, and lies about 15μ from the posterior end. The ***undulating membrane*** is plainly visible. The free flagellum appears to be short, 2μ or so, but this may be due to deficient staining. The ***nucleus*** is roundish, and stains a pale red. It lies on the outside curve of the body

midway between the ends; in diameter about 3μ, it varies with the degree of flattening of the specimen on the slide. The *cytoplasm* stains a dark blue with a few clear areas located in the anterior and posterior ends and around the kinetoplast. The general appearance is granular with some vacuoles. A number of specimens show *myonemes*, the number of lines varying from five to eight according to the flattening of the specimen.

Many trypanosomes are polymorphic. In the absence of cultures and in view of the fewness of specimens the identification here must be considered incomplete. A very narrow range of variation of *Trypanosoma gallinarum* Bruce *et al.* (1), would include all the specimens seen, and to this species it must be temporarily referred.

Host Records

Bonasa umbellus. The specimens described and the figures are from ruffed grouse No. 176, juvenile male, Brule Lake, July 10, 1934.

Other Brule Lake records are No. 168, adult male, June 7, 1934, and No. 169, adult male, June 14, 1934.

Canachites canadensis, spruce grouse No. 124, Frank's Bay, May 15, 1933.

Microfilariae

Microfilariae have been found in two instances, both sets being similar. The adult worms were not found in spite of the most careful dissection. Larvae were not abundant.

Host Records

Ruffed grouse No. 168, adult male, Brule Lake, June 7, 1934.

Spruce grouse No. 124, adult female, Frank's Bay, May 15, 1933.

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VARIATION IN WEIGHT OF SOME INTERNAL ORGANS OF THE DOMESTIC FOWL *(GALLUS GALLUS)*[1]

By J. W. Hopkins[2] and J. Biely[3]

Abstract

One hundred apparently normal yearling single-comb White Leghorn hens were subjected to post mortem examination, including weighing of the liver, kidneys and spleen. The average weight of these organs (excluding two individuals possessing only one kidney) constituted 1.89, 0.64 and 0.12% of the average total live weight. Single organs, particularly the spleen, were relatively more variable than total body weight.

The weight of the organs from individuals of similar total live weight varied markedly, but, on the whole, the larger birds had somewhat larger organs. As the size of bird increased, however, the average percentage of the total weight due to liver, kidneys or spleen decreased.

There was a moderate but significant correlation ($r = +0.41$) between the weights of liver and kidneys from birds of a specified total weight, but no evidence of association between weight of kidneys and spleen weight.

Introduction

In the course of a previously reported study of normal and pullorum-disease-infected fowls (1), 100 apparently normal yearling single-comb White Leghorn hens were subjected to post mortem examination, including weighing of the liver, kidneys and spleen. A statistical study has been made of the recorded weights of these organs, both individually and in relation to the weight of the bird as a whole.

Previous quantitative anatomical investigations of the fowl seem to have been concerned chiefly with tracing the relative growth of the various systems and organs. Thus Latimer (2) found that the average weight of liver and kidneys, relative to that of the body as a whole, decreased from early maxima of 6.2 and 2% to approximately 2.5 and 0.6% in the adult single-comb White Leghorn (including both sexes). The actual weight of the liver increased noticeably in the older birds however, especially in the fat hens. The relative weight of the spleen (3) also decreased with age, from an early maximum. Analogous results are reported by Mitchell, Card and Hamilton (4) from successive groups of five White Plymouth Rock cockerels, pullets and capons selected from a large flock. As the approximate slaughter weight of cockerels increased from one to seven pounds, the percentage of that weight due to liver, kidneys and spleen decreased from 3.7, 1.1 and 0.21 to 1.3, 0.39 and 0.11 respectively. The liver, kidneys and spleen of approximately 5-lb. pullets formed on the average 1.9, 0.62 and 0.21% of the total bird weight.

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, and the Department of Poultry Husbandry, University of British Columbia, Vancouver, B.C.

[2] *Biologist, National Research Laboratories.*
[3] *Research Assistant, Department of Poultry Husbandry, University of British Columbia.*

In both of the foregoing investigations the size of the organs of individual birds of the same age or weight varied considerably, but no quantitative study of such differences was made. Souba (5) on the other hand specifically investigated this variation, using single-comb White Leghorn cockerels 100 to 120 days of age, *i.e.*, in the phase of rapid growth associated with puberty. The coefficient of variation of total body weight proved to be 19.0, whereas the coefficients of variation of the kidneys, liver and spleen were 19.2, 21.9 and 35.9 respectively. There was also a highly significant correlation between total bird weight and the weight of individual organs. These results, as mentioned, were obtained from actively growing birds. The authors are not aware of any similar biometric data respecting mature fowls.

Material and Methods

The 100 yearling hens furnishing the anatomical data were secured from several commercial flocks. They were kept together in one house, under uniform conditions of feeding and management, for about one month prior to slaughter, and throughout this period of observation all appeared to be healthy and vigorous. Only a few were laying; about 25 were in moult, and the remainder were just beginning to shed their feathers.

Killings were carried out between October 18 and November 4, 1933, at which time the birds were approximately 17 to 18 months old. Those selected for slaughter on any given day were transferred from the laying house to exhibition cages, containing food and water, in the laboratory. Immediately prior to killing, all birds were weighed, the live weight including the food present in the digestive tract. Whilst the body was still warm, the liver, spleen and kidneys were cut out in the order named and weighed as rapidly as possible, in order to minimize losses due to evaporation. The gall bladder was removed from the liver, and the ureters and excess fat from the kidneys; both kidneys were weighed together. The weights obtained are shown in Table I.

As stated, a post mortem examination was made of every carcass. The internal organs appeared to be normal in every respect save that two birds had one kidney only, a condition not previously observed in any of the more than 1000 specimens examined in this laboratory. These were excluded from subsequent calculations. Only a few cases of infestation of the intestine with roundworms or tapeworms were observed, and in no instance did these parasites appear to be present in sufficiently large numbers to affect general health. The birds may therefore be regarded as comprising a group representative of flocks kept in good health and free from parasitic or infectious disease.

Variation in Weight of Bird and Organs

The total live weight and the weight of the organs of the 98 individual birds are shown graphically in Fig. 1. Total weights recorded vary from 1200 to 2175 gm., the average being 1590.7 gm. Individual livers fall between 21.5 and 52.3 gm., with an average of 30.00 gm.; kidneys between 6.9 and

TABLE I

LIVE WEIGHT AND WEIGHT OF INTERNAL ORGANS OF HENS

Total live weight, gm.	Weight of liver, gm.	Weight of kidneys, gm.	Weight of spleen, gm.	Total live weight, gm.	Weight of liver, gm.	Weight of kidneys, gm.	Weight of spleen, gm.
1200	26.9	8.06	2.01	1591	35.6	9.96	1.32
1212	29.4	9.08	2.10	1593	32.4	14.60	1.70
1216	24.1	8.11	1.14	1594	41.5	11.74	1.67
1230	29.5	8.15	2.31	1600	31.7	13.83	1.34
1272	24.7	9.58	1.55	1600	29.7	7.80	2.35
1307	26.3	10.37	1.34	1615	27.0	10.38	1.68
1320	30.0	8.14	2.66	1616	25.2	8.62	1.67
1328	37.7	10.92	1.42	1618	27.7	10.12	1.90
1340	22.6	7.45	1.16	1620	40.3	11.45	1.74
1340	28.2	8.52	2.88	1635	32.3	10.06	2.60
1372	26.0	7.02	1.04	1640	29.0	8.93	1.20
1372	26.3	7.41*	1.62	1645	25.9	10.08	1.26
1375	22.8	8.57	1.19	1654	25.8	10.52	1.28
1380	30.0	8.35	2.88	1658	23.8	9.14	1.02
1390	28.3	11.00	1.27	1664	28.2	8.27	2.12
1400	23.1	9.46	1.29	1669	34.6	9.99	3.40
1403	26.1	8.52	2.63	1672	27.3	8.55	1.46
1404	27.5	11.51	2.34	1674	39.1	8.30	2.06
1412	28.3	10.14	1.84	1675	44.7	13.01	1.97
1415	22.9	8.99	1.52	1680	32.2	11.60	1.28
1416	39.6	10.84	1.68	1684	34.9	10.94	2.24
1417	29.5	9.35	1.07	1690	24.9	6.91	1.48
1420	23.4	10.22	1.20	1715	32.5	10.82	1.94
1430	27.3	10.37	1.88	1727	29.8	11.67	1.53
1430	26.6	9.08	1.87	1728	32.8	14.07	2.16
1436	29.3	10.48	1.35	1730	27.4	10.18	2.10
1438	33.3	11.58	4.26	1730	32.2	12.29	1.90
1445	36.2	10.99	2.61	1735	25.6	10.61	1.33
1450	29.3	9.80	1.87	1735	26.4	9.03	1.88
1454	31.0	8.88	1.14	1739	31.7	9.14	1.21
1455	32.3	8.26	1.48	1740	22.8	11.22	2.39
1455	41.2	14.54	0.90	1748	38.7	11.04	3.14
1460	28.3	8.66	1.28	1750	28.1	9.55	1.51
1461	35.0	12.69	1.49	1777	27.4	10.26	2.03
1496	23.4	9.53	2.37	1782	29.8	13.17	2.18
1505	31.6	8.46	2.14	1787	27.6	10.39	1.60
1512	25.9	9.23	1.67	1788	34.1	9.09	2.68
1518	26.7	10.28†	1.47	1789	25.2	7.46	1.33
1520	28.9	9.68	1.18	1800	37.5	13.18	1.79
1530	30.9	10.54	1.05	1830	27.5	10.86	2.68
1544	30.4	8.11	1.48	1831	35.2	12.02	1.93
1550	29.8	9.45	1.62	1844	29.5	8.39	1.70
1557	34.1	14.60	1.94	1851	29.8	11.44	3.02
1559	27.3	8.39	1.46	1880	35.2	13.38	2.90
1566	25.5	10.02	1.98	1900	28.5	10.62	1.93
1570	31.2	9.38	3.10	1903	27.2	10.12	2.50
1573	26.0	10.32	3.68	1960	31.8	11.45	2.18
1583	30.6	8.35	1.33	2000	29.2	11.05	2.84
1587	30.6	14.54	1.65	2100	36.1	11.08	3.12
1591	21.5	11.52	1.58	2175	52.3	13.26	1.74

* *Left kidney only; no right one.*

† *Right kidney only; no left one.*

14.6 gm., with an average of 10.22 gm.; and spleens between 0.90 and 4.26, with an average of 1.886 gm. The average liver, kidney and spleen weights constitute 1.89, 0.64 and 0.12% of the average total live weight. The standard deviation of total live weight was found to be 195.0 gm., or 12.3% of the mean; and that of the liver, kidneys and spleen to be 5.27, 1.79 and 0.65 gm. or 17.6, 17.6 and 34.5% of the mean respectively. Single organs,

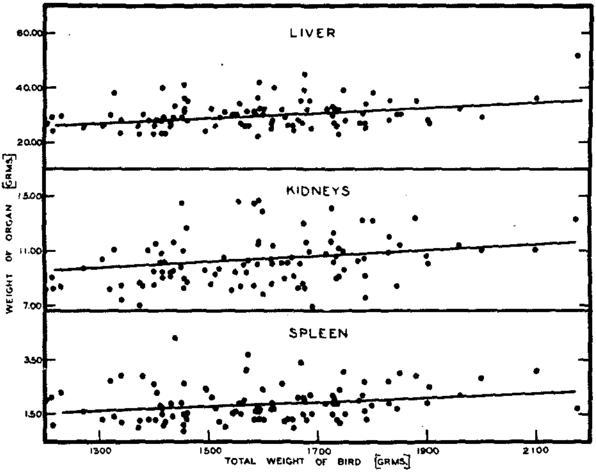

FIG. 1. *Live weight and weight of certain internal organs of 98 yearling single-comb White Leghorn hens.*

and particularly the spleen, are thus relatively more variable in weight than the body as a whole, as in the case of the growing cockerels studied by Souba (5). It might be thought that the high relative variability of the observed spleen weights was occasioned by operational injuries or small amounts of adhering tissue exerting an appreciable influence on the total weight of so small an organ; but in fact the spleen was the easiest of the three organs to excise, as it is encapsulated and can be removed as a whole. The method of killing (breaking the neck) may however have resulted in some of the spleens being more engorged with blood than others.

It will be evident from Fig. 1 that there is a marked variation in the weight of, for example, the liver from birds of similar total weight; and this is also true of the kidneys and spleen. Nevertheless the size of these organs is not wholly independent of the size of the bird. Computation of the coefficient of correlation between the total weight and liver, kidney and spleen weight

yields values of $r=+0.32$, $+0.34$ and $+0.22$ respectively. Although certainly not indicative of any high degree of association, the lowest of these values exceeds the 5% point (0.20) and the other two exceed the 1% point (0.26) for a sample of the size considered, and it may be concluded that there is a tendency for the larger birds to have on the average larger organs. The correlation is, however, much more moderate than that encountered by Souba (5), possibly because differences in the gross weight of mature hens are due not only to differences in the development of the individual as a whole, but also to variations in the amount of muscular tissue or fat and particularly in the condition of the ovary and oviduct from bird to bird, which have probably little relation to organ development.

The regression equations, giving the average organ weight associated with any specified bird weight, in gm., are:

$$\begin{aligned} \text{Liver wt.} &= 16.34 + 0.00859 \text{ total wt.} \\ \text{Kidney wt.} &= 5.19 + 0.00316 \text{ total wt.} \\ \text{Spleen wt.} &= 0.718 + 0.000734 \text{ total wt.} \end{aligned}$$

The course of these functions is illustrated by the regression lines in Fig. 1. All are of the form $y=a+bx$, where a is a positive quantity; the ratio y/x, that is of any organ weight to total weight, will therefore diminish with increasing total weight. This is illustrated in Table II, in which the average organ weights of 1200-, 1700- and 2200-gm. birds, calculated from the foregoing formulas, are expressed as a percentage of the respective total live weights. It is interesting to observe this tendency in individual fowls of the same age group, as well as in the averages of different age groups dealt with by the investigators previously mentioned.

TABLE II

WEIGHT OF ORGANS AS PERCENTAGE OF TOTAL LIVE WEIGHT

Total live weight, gm.	Liver, %	Kidneys, %	Spleen, %
1200	2.22	0.75	0.13
1700	1.82	0.62	0.12
2200	1.60	0.55	0.11

The regression equations may also be used to compute the standard deviation of the individual organ weights from the appropriate regression line. In this way, allowance is made for the increase in average organ weight with total live weight, and an estimate of the variation in weight of the organs of birds of any specified total weight is provided. Owing to the low degree of correlation these adjusted values differ but little from those neglecting the regression. They are 5.02, 1.69 and 0.638 gm., or 16.7, 16.6 and 33.8% of the mean in the case of liver, kidneys and spleen respectively.

Correlation between the weight of different organs from the same bird may also be considered briefly. Neglecting the regression on total body weight, the correlation coefficient between liver weight and weight of kidneys is found to be $+0.48$, and that between weight of kidneys and spleen weight to be $+0.11$. A part of this association however is due to the fact that on the

average all the organs considered tend to be larger in the larger birds. When due allowance is made for the influence of total weight, the above coefficients are reduced to +0.41 and +0.03 respectively. There would thus appear to be a moderate but significant correlation between the weight of liver and kidneys from birds of a specified total weight, but no evidence of association between weight of kidneys and spleen weight.

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THE HETEROTHALLISM OF *PANAEOLUS SUBBALTEATUS* BERK., A SCLEROTIUM-PRODUCING AGARIC[1]

By Harold J. Brodie[2]

Abstract

Panaeolus subbalteatus, an uncommon coprophilous agaric, has been grown in single-spore culture on malt-extract agar.

The fungus is heterothallic, exhibiting four sexual groups and a remarkable regularity in its pairing reactions.

Both haplophytes and diplophytes produce sclerotia of a striking greenish-blue color. These sclerotia are capable of producing mycelium, even after they have been dried for some weeks, but do not give rise to fruit bodies.

The haplophyte is distinguishable macroscopically as well as microscopically from the diplophyte.

The mycelia do not exhibit the phenomenon of mutual aversion or *barrage*.

Panaeolus subbalteatus Berk. is a rather uncommon coprophilous agaric which may be found in gardens and on lawns where manure has been used to enrich the soil. A description of the species has been given by A. H. Smith (3) who was the first to record its occurrence in the state of Michigan. The fungus was found on the campus of the University of Michigan at Ann Arbor in October 1933. It was identified by Dr. Smith who kindly gave several specimens to the writer.

From a deposit of basidiospores shed by a single fruit body, a series of 25 monosporous cultures was obtained by the sprayed-plate method (2). The black apiculate basidiospores (measuring $8 \times 12\ \mu$) germinated in 12 hr. on malt-extract agar. The haploid mycelia developed quite slowly, requiring two weeks to grow from the centre to the periphery of an agar plate 9 cm. in diameter.

Owing to the pressure of culture work with other species of agarics, the writer was unable to give further attention to the *Panaeolus* until the month of January. The haploid mycelia were kept in a condition of vigorous growth in the interim by making transfers once every month.

One of the most striking characters of the mycelium of *Panaeolus subbalteatus* in laboratory culture, is the abundant production of sclerotia. A mycelium freshly transferred to a Petri dish of malt agar or of other medium grows for about five days, producing only ordinary vegetative hyphae. The mycelium then becomes laden with numerous large drops of watery exudate and at the same time small masses of mycelium begin to accumulate on the older portions of the thallus. The sclerotial primordia are of a striking greenish-blue color. Upon discovering nodules of bright greenish-blue lying upon the white mycelium, in all the culture tubes, the writer's first impression was that the entire stock had become contaminated with a *Penicillium*. Closer examination revealed the fact that the nodules were in reality sclerotia,

[1] *Manuscript received January 19, 1935.*
Contribution from the Department of Botany, University of Toronto, Toronto, Canada.
[2] *Fellow of the National Research Council (United States) at the University of Toronto.*

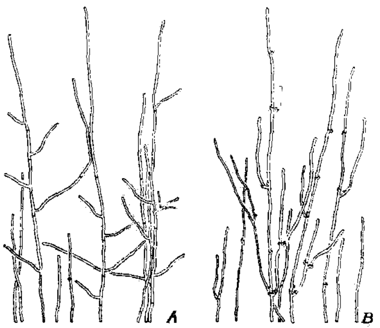
spherical in shape and varying in size from 1 to 4 mm. with an occasional one as much as 6 mm. in diameter. Plate I, Fig. 2, shows the appearance of the sclerotia.

Cultures varied in the tendency which they exhibited to produce sclerotia. Occasionally only a certain sector of a culture on an agar plate would produce sclerotia, the remainder of the mycelium being fluffy and devoid of them. Such a culture is shown in Plate I, Fig. 4, *B*.

The sclerotia soon became hard and darker in color. Some were allowed to dry for a month and when these were placed on a freshly poured agar plate they germinated to give rise to haploid mycelium. Frequently sclerotia are known to give rise to fruit bodies but the writer was able to obtain only vegetative mycelium by allowing the sclerotia to "germinate".

On January 9, 1934, the haploid mycelia were paired in tubes in all possible combinations and two weeks later the pairings were examined. In some tubes diploid mycelium had made its appearance and could be distinguished from the haploid mycelium macroscopically. The hyphae of the diploid mycelium are slightly straighter and coarser than the hyphae of the haploid mycelium. The diploid mycelium not only appeared along the line of contact of the two haplophytes but also developed from the haploid mycelium, indicating that in *Panaeolus subbalteatus* diploidization is similar to what has been described by Buller (1) for *Coprinus lagopus*.

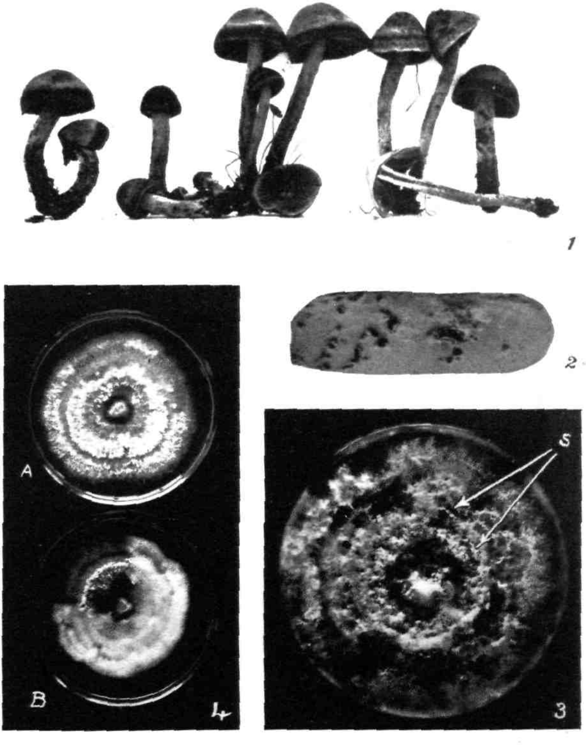
The haploid mycelium is composed of fine hyphae whose average diameter is 4 μ. The angle of branching tends to be rather wide. The hyphae of the diploid mycelium are coarse (about 5–6 μ in diameter) and branch at an angle considerably smaller than that of the haploid mycelium. Although the haplophyte grows somewhat faster than the diplophyte, the difference in growth rate is not great and was not measured. The clamp connections on the diploid mycelium are small, the hook cell being much less arched than in some fungi such as *Coprinus lagopus* (1, p. 202). The two kinds of mycelium are contrasted in Text-fig. 1, *A* and *B*.



TEXT-FIG. 1. *The haploid (A) and diploid (B) mycelia of Panaeolus subbalteatus, each growing in a hanging drop of malt-extract agar. Magnification, 90.*

Sclerotia appeared on all diploid as well as haploid mycelia. The sclerotia occurring on the diplophytes gave rise to diploid mycelium and never to haploid.

PLATE I



Panaeolus subbalteatus. FIG. 1. *Specimens collected by Dr. A. H. Smith on the campus of the University of Michigan, June 1934, and photographed by him. The specimens are smaller than some collected in 1933 and probably represent a dry weather form of the fungus. Natural size.* FIG. 2. *Agar and haploid mycelium withdrawn from culture tube, showing sclerotia. Natural size.* FIG. 3. *A diploid mycelium three weeks old showing the sclerotia, s. Two-thirds natural size.* FIG. 4. *B, a haploid mycelium, No. 2 (Ab), two weeks after having been planted on an agar plate. One sector only of the mycelium is producing sclerotia which are seen as a dense white arc half-way between the centre and the periphery of the mycelial mat; A, a diploid mycelium, No. 2 + 6, the same age as the haplophyte and also producing sclerotia. One-half natural size.*

Neither haplophyte nor diplophyte showed any tendency to produce carpophores in culture, nor did either produce the oidia which are so frequently found in hymenomycetous fungi.

In Text-fig. 2 the results of the pairings are given in tabular form, a plus sign indicating the presence of clamp connections and a minus sign their absence. In the long series of pairings there were no irregularities, the mycelia falling perfectly into four groups.

		AB						*ab*							*Ab*					*aB*						
		1	4	10	11	20	25	5	8	9	13	17	19	21	2	3	7	12	15	6	14	16	18	22	23	24
AB	1		−	−	−	−	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	4	−		−	−	−	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	10	−	−		−	−	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	11	−	−	−		−	−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	20	−	−	−	−		−	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	25	−	−	−	−	−		+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
ab	5	+	+	+	+	+	+		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	8	+	+	+	+	+	+	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	9	+	+	+	+	+	+	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	13	+	+	+	+	+	+	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	17	+	+	+	+	+	+	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−
	19	+	+	+	+	+	+	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−
	21	+	+	+	+	+	+	−	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−
Ab	2	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	+	+	+	+	+	+	+
	3	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	+	+	+	+	+	+	+
	7	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	+	+	+	+	+	+	+
	12	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	+	+	+	+	+	+	+
	15	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		+	+	+	+	+	+	+
aB	6	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+		−	−	−	−	−	−
	14	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−		−	−	−	−	−
	16	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−		−	−	−	−
	18	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−		−	−	−
	22	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−		−	−
	23	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−	−		−
	24	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−	−	−	

TEXT-FIG. 2. *The heterothallism of Panaeolus subbalteatus demonstrated by pairing monosporous mycelia in all possible combinations.*

In none of the pairings between haploid mycelia of *Panaeolus subbalteatus* was there exhibited the phenomenon of aversion or "barrage" which has been described by Vandendries and Brodie (7) for *Lenzites betulina.*

From the results of the pairing experiment we may conclude that *Panaeolus subbalteatus* is heterothallic and that it exhibits four sexual groups*. As far as the writer is aware, the sexuality of only two other species of *Panaeolus*

For possible future reference, cultures of the haploid mycelia Nos. 1 (AB), 5 (ab), 2 (Ab) and 6 (aB) have been deposited in the Centraal Bureau voor Schimmelcultures at Baarn, Holland, as well as the two diploid mycelia Nos. 1 + 5 and 2 + 6.

has been ascertained. *P. campanulatus* Fr., was shown to be tetrapolar in 1923 by Vandendries (4) and the same investigator later (5) reported *P. papilionaceus* Fr., as being tetrapolar. Both of these species exhibit in some degree the phenomenon of "barrage" or repulsion between certain mycelia. Vandendries (6), reporting on his attempts to obtain the germination of spores of various species of *Panaeolus*, lists the following four as germinable: *P. campanulatus*, *P. fimicola*, *P. separatus* and *P. papilionaceus*. He was unable to germinate the spores of *P. sphinctrinus*.

In concluding, the writer wishes to express his gratitude to Dr. A. H. Smith for the gift of material from which the research recorded above was made and for notes regarding the species.

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THE OIDIA OF *PSILOCYBE COPROPHILA* AND THE PAIRING REACTIONS OF MONOSPOROUS MYCELIA[1]

By Harold J. Brodie[2]

Abstract

By isolating thirty monosporous mycelia and pairing them, the agaric *Psilocybe coprophila* Fr. has been shown to be *heterothallic* and bipolar, confirming the report of Miss Kathryn Gilmore (1926). Contrary to the statement of Miss Gilmore, no oidia were found on the diploid mycelia although they are abundant on the haplophytes. The diplophytes are frequently "impure", hyphae devoid of clamp connections and are found intermingled with hyphae bearing clamp connections. The possibility of the clamp-connection-free hyphae being haploid and of the development of oidia on these haploid hyphae is suggested as an explanation of the statement of Miss Gilmore that oidia occur on the diplophyte of this fungus.

No mutual repulsion between haploid mycelia was observed. This is negative evidence in support of the prediction of Vandendries and Brodie that their *barrage* phenomenon would not be found in bipolar but only in tetrapolar hymenomycetes.

Introduction

An interesting account of her study of *Psilocybe coprophila* in culture was published in 1926 by Miss Kathryn Gilmore (5). In connection with researches concerning the function of the oidia of the Hymenomycetes, several points in Miss Gilmore's paper were of particular interest to the writer when he first read it in 1930.

A rather extensive study of the life history and sexuality of *Coprinus lagopus* was being carried on at that time in the laboratory of Professor A. H. R. Buller at Winnipeg. While working with Dr. Buller, the writer (1, 2) studied the function of the oidia of *Coprinus lagopus*. It was shown that: (i) the oidia are produced in little masses in drops which crown the ends of aerial oidiophores; (ii) the oidia are borne on haploid but never on diploid mycelia; (iii) insects can transfer oidia of one sexual strain to mycelia derived from spores of the opposite sexual strain; and (iv) the oidia so transferred may germinate, fuse with the mycelia to which they have been brought, and cause these to become diploid. The conclusion that only the haploid mycelia of the heterothallic hymenomycetous fungi produce oidia seemed to be strengthened by an examination of several other species of agarics: diploid mycelia bearing oidia did not come under observation.

Miss Gilmore stated that, in *Psilocybe coprophila*, oidia are developed not only on the haploid mycelia but also on the diploid. The oidia produced by the haplophytes were found to germinate and give rise to haploid mycelia of the same constitution as the parent culture. But with regard to the oidia developed on a diploid mycelium (produced by pairing two haploid mycelia

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of known sexual strain), Miss Gilmore concluded that: "They sometimes germinate to form diploid mycelia; at other times they germinate to form haploid mycelia, segregation of the nuclei occurring before the oidia are produced." Of seventeen single-oidium cultures, isolated from a diploid mycelium, sixteen were haploid; only one was diploid. Concerning this one Miss Gilmore wrote: "It might have originated from a small bit of diploid mycelium or two unlike oidia erroneously taken for a single germinating oidium.".

Because Miss Gilmore had reported finding oidia on the diplophytes of *Psilocybe coprophila* whereas the writer found no oidia on the diplophytes of *Coprinus lagopus* and other *Coprini* and, further, because of some uncertainty in Miss Gilmore's conclusions it seemed advisable to make a fresh study of cultures of the *Psilocybe*. Unfortunately Miss Gilmore's cultures were no longer available and it was not until recently that an opportunity for continuing the study presented itself.

In the meantime the writer (3) examined more than a dozen species of agarics in the genera *Collybia*, *Coprinus*, *Corticium*, *Polystictus*, *Hypholoma* and *Lenzites*. From this research the following conclusions were drawn: (i) that heterothallic hymenomycetes as a rule produce oidia on the haploid mycelium but not on the diploid: and (ii) that, as far as is known, homothallic hymenomycetes do not produce oidia.

An extensive study of *Collybia velutipes* (3) has revealed the fact that oidia are developed on the diplophyte as well as on the haplophyte in this species. These oidia are *haploid* and are borne on haploid branches arising from the diploid mycelium. Such haploid branches are produced by the separation of the nuclei of the dicaryon either by the migration of the nuclei into different branches or, rarely, by the abnormal elongation of the hook cell during the process of the formation of clamp connections. Half the oidia borne on the diplophyte are thus of the same sex as one of the parent mycelia originally used to obtain the diplophyte and half are of the same sex as the other parent mycelium.

Examining Miss Gilmore's results in the light of the above discovery it appeared possible that discounting certain errors (*e.g.*, two unlike oidia erroneously taken for a single oidium), the situation in *Psilocybe coprophila* might fall into line with that in *Collybia velutipes*, *i.e.*, that segregation of the nuclei occurs before the oidia are produced.

That true diploid oidia occur on the diplophytes of *Pholiota aurivella* Batsch., was clearly demonstrated in 1932 by Vandendries and Martens. This species alone, of over twenty referred to in the literature (3) is definitely known to produce diploid oidia. It appears that oidia are most commonly associated with the haploid mycelia of heterothallic species.

There were, therefore, several reasons why a fresh study of *Psilocybe coprophila* might be profitable It was necessary to ascertain: (i) whether or not oidia actually do occur on the diploid mycelium of *P. coprophila;* and (ii) whether the oidia, if present, are haploid or diploid.

There was still another reason for the advisability of examining cultures of *P. coprophila*. In 1933 (7), in collaboration with Dr. René Vandendries, the writer described the phenomenon of mutual repulsion between certain haploid strains of *Lenzites betulina*, *e.g.*, between (*ab*) and (*aB*) or between (*Ab*) and (*AB*). The repulsion results in a zone barren of mycelium between the two haplophytes which have been paired. The phenomenon has been given the name *barrage*. In defining the term, Vandendries and Brodie declared that repulsion is manifest only in tetrapolar species when the *b* factor is possessed in common by the two haplophytes and the *a* factor differs in each. *A priori* there should be no mutual aversion between haplophytes of a bipolar species where only one pair of factors regulates the pairing of mycelia. *Psilocybe coprophila* had been shown by Miss Gilmore to be bipolar and it appeared to be an excellent subject for testing the correctness of the assumption that "barrage" is not manifest in a bipolar fungus.

The Haploid Mycelia

Several carpophores found on cow dung in Ann Arbor, Michigan, were identified by Dr. A. H. Smith as *Psilocybe coprophila* Fr. (in the sense of Ricken). Dr. Smith kindly gave the specimens to the writer and 30 monosporous cultures were obtained from the spores shed by a single fruit body. The sprayed-plate method of Kauffman (6) was used in isolating the spores.

The mycelia were grown on malt-extract agar (15 gm. malt extract of Merck & Co., and 15 gm. agar to one litre of distilled water), and on the "*IIa*" agar recommended by Miss Gilmore (5, p. 421). The latter precaution was taken to ensure conditions identical with those under which Miss Gilmore grew her cultures. Growth was good on both media, the mycelia forming dense white mats (Plate I, Fig. 1). All the cultures on malt-extract agar possessed a very characteristic odor resembling the odor of sweet corn when cooking. Some of the cultures produced dark brown sclerotia about 4 mm. in diameter and on many of the haplophytes there appeared rudiments of fruit bodies which, however, did not develop into perfect fruit bodies.

All haplophytes produced oidia abundantly. Special coiled hyphae by segmentation give rise to the allantoid oidia which are about 1.5 μ in diameter and 4–8 μ in length.

Miss Gilmore reported (5, p. 423) that of 24 single-spore cultures which she obtained, 23 were haploid and one diploid. All the monosporous mycelia isolated in the present study were haploid, none of the mycelia bearing clamp connections.

The Pairing of Monosporous Mycelia

When the haplophytes were a month old they were paired in tubes in all possible combinations, 436 pairings in all having been made. Clamp connections appeared on some of the mycelia two days after the pairing but the entire series was not examined critically until the end of the second week.

In Text-fig 1 are presented the results of the experiment. In this table a plus sign indicates the presence of clamp connections on the paired mycelia, a minus sign their absence.

		A																	*a*												
		1	*4*	*5*	*6*	*11*	*12*	*13*	*14*	*15*	*18*	*19*	*21*	*22*	*23*	*24*	*25*	*30*	*2*	*3*	*7*	*8*	*9*	*10*	*16*	*17*	*20*	*26*	*27*	*28*	*29*
	1		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	4	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	5	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	6	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	11	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	12	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	13	−	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	14	−	−	−	−	−	−	−		−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
A	*15*	−	−	−	−	−	−	−	−		−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	18	−	−	−	−	−	−	−	−	−		−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	19	−	−	−	−	−	−	−	−	−	−		−	−	−	−	⊖	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	21	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	22	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	23	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	24	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
	25	−	−	−	−	−	−	−	−	−	−	⊖	−	−	−	−		−	+	+	+	+	+	+	+	+	+	+	+	+	+
	30	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		+	+	+	+	+	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		−	−	−	−	−	−	−	−	−	−	−	−
	3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−		−	−	−	−	−	−	−	−	−	−	−
	7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−		−	−	−	−	−	−	−	−	−	−
	8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−		−	−	−	−	−	−	−	−	−
	9	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−		−	−	−	−	−	−	−	−
	10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−		−	−	−	−	−	−	−
a	*16*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−		−	−	−	−	−	−
	17	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−		−	−	−	−	−
	20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−		−	−	−	−
	26	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−		−	−	−
	27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−		−	−
	28	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−		−
	29	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	

TEXT-FIG. 1. *Table showing the results of pairing in all possible combinations of 30 monosporous mycelia of Psilocybe coprophila.*

It will be seen that the mycelia fall into two sexual groups, seventeen in *one group and thirteen in the other.* The fungus is therefore clearly bipolar as has been shown by Miss Gilmore.

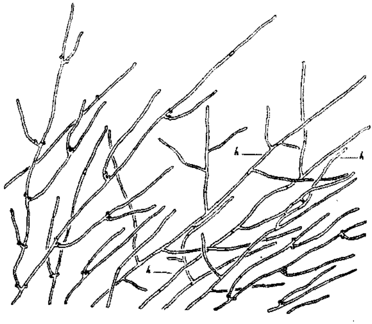
In none of the 435 pairings was there any sign of mutual repulsion between the haploid mycelia. In a few tubes there were lines of demarcation between the individual mycelia but these lines disappeared as intermingling of the mycelia became complete. Inasmuch as not all tetrapolar species of the Hymenomycetes exhibit the barrage phenomenon, its nonappearance in the present example of a bipolar species is only negative evidence in support of the statement of Vandendries and Brodie that barrage is not manifest in a bipolar hymenomycete.

In the table of pairings (Text-fig. 1), the reaction of mycelium No. 19 with No. 25 is indicated as exceptional by means of a circle around the minus sign. The mycelium resulting from the pairing bore a few false clamp connections. However when a confirmatory pairing was made a few days later, the irregularity did not reappear. The cause of the temporary irregularity is unknown.

The Diploid Mycelium

In none of the 221 diplophytes were any oidia to be found, a result which fails to confirm the statement of Miss Gilmore that oidia occur on the diploid mycelium in this species.

Some of the diplophytes exhibited "patchiness", *i.e.*, the mycelium was uneven, parts of it being white and fluffy, other parts less fluffy. Two patchy diplophytes are shown in the photographs reproduced in Plate I, Fig. 2. These mycelia when examined microscopically were revealed as including some haploid hyphae along with the diploid. The diploid mycelium No. 1 + 2 is illustrated in Text-fig 2, and it will be seen that there are entire hyphae which are haploid, at least judging from the fact that the cross-walls of these hyphae are devoid of clamp connections*. It was estimated that in such impure diplophytes not more than 10% of the hyphae are haploid.



TEXT-FIG. 2. *Hyphae at the periphery of the diploid mycelium No. 1 + 2, growing in a hanging drop of malt-extract agar. Haploid hyphae (h) are seen growing among the diploid hyphae. Drawn with the aid of the camera-lucida. Magnification 90.*

To account for the appearance of haploid hyphae among the diploid,

**Positive evidence that these clamp-connection-free hyphae are actually haploid will have to be obtained by a cytological examination. This will be undertaken by the writer in due course. It has been shown (3) that similar hyphae occur on the diploid mycelium of Collybia velutipes and that they contain but one nucleus in each cell. Hence it seems highly probable that the clamp-connection-free hyphae in Psilocybe coprophila are really haploid.*

two possible explanations occur to the writer: (i) when the two haplophytes were paired, diploidization was in some way incomplete so that certain hyphae remained haploid; or (ii) diploid hyphae gave rise to haploid hyphae by the separation of the nuclei of a dicaryon.

Buller (4, p. 248) has described the occurrence of irregularities in the appearance of the diploid mycelium of *Coprinus lagopus* and has used the word "patchy" to describe the mycelia. The patchy mycelia of Buller were derived by the pairing of a diploid mycelium with a haploid mycelium, the two being theoretically incompatible. The diploid mycelium is able to effect an imperfect diploidization of the haplophyte, probably by both nuclei of the dicaryon of the diploid mycelium passing into the haploid mycelium. This incomplete diploidization is not strictly comparable to the occurrence of patchy diplophytes in *Psilocybe coprophila*, but there may be some similarity in the two phenomena.

Had diploidization in *P. coprophila* been incomplete, it should have been possible, by making fresh transfers from the diploid mycelia and taking care to select only mycelium bearing clamp connections, to obtain "pure" diploid mycelium. This method of transfer was actually used for each of the four diploid mycelia Nos. 1 + 2, 1 + 8, 2 + 4, 3 + 4; but when the mycelia which developed on the fresh plates were examined a few days later, haploid hyphae were still present among the diploid.

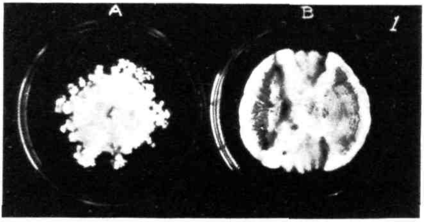
If the first hypothesis be accepted, we must assume that the hyphae which were left haploid after diploidization had taken place were altered in such a way that they were no longer capable of being diploidized by any of the other hyphae present in the culture.

When mycelium free from clamp connections was isolated from an impure diplophyte, the mycelium obtained was not haploid but again an impure diplophyte.

The second hypothesis seems more probable. As stated, it was found (3) that in *Collybia velutipes* diploid hyphae actually do give rise to haploid by the isolation of the nuclei of the dicaryon in separate hyphal branches. In cultures of *C. velutipes* it was quite easy to find the origin of the haploid branches by examining a culture of the diploid mycelium growing in a Van Tieghem cell. Bits of diploid mycelium of *Psilocybe coprophila* were planted on agar drops in Van Tieghem cells and allowed to develop. In all cultures examined in this manner, the haploid hyphae appeared to originate in the central mass of mycelium. They arose so far from the younger and less dense portions of the mycelia that it was quite impossible to form any clear idea as to how they had originated. However, it is quite possible that their mode of formation is the same as in *Collybia velutipes*.

The haploid hyphae occurring on the diplophytes of *C. velutipes* bear oidia. Thinking that a similar situation might obtain in *Psilocybe coprophila*, a very careful search through all the diploid mycelia was again made with the result that no oidia were found. It is not improbable, however, that the

PLATE I



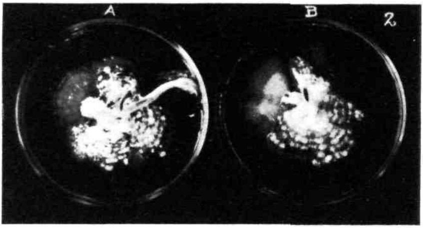


FIG. 1. *Two haploid mycelia of Psilocybe coprophila two weeks old; A, No. 1, B, No. 2. One-half natural size.*

FIG. 2. *Two patchy diplophytes: A, No. 1 + 2, B, No. 1 + 8. In A is shown an imperfect carpophore. One-half natural size.*

residual haploid hyphae occurring along the diploid hyphae might, under certain circumstances, produce oidia. Unless the mycelia were examined exceedingly carefully, the observer might easily be led to believe that the oidia were borne on diploid mycelium properly so-called.

On Plate 33, Fig. 9 of her article, Miss Gilmore illustrates oidia borne on a mycelium bearing clamp connections. The writer has failed to find anything resembling this in his cultures. There is, nevertheless, no reason for not supposing that the oidia in question might have developed on *haploid* mycelium which *later* became diploidized and bore clamp connections.

On many of the diplophytes there were produced fruit bodies, some of which developed normally, others of which were abnormal (Plate I, Fig. 2) as has been reported by Miss Gilmore.

Acknowledgment

The writer wishes to thank Dr. A. H. Smith for providing him with the carpophores of *Psilocybe coprophila** and for identifying them.

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* *Cultures Nos. 1, 2, 3, 4 and 1 + 2 have been deposited in the Centraal Bureau voor Schimmelcultures at Baarn, Holland.*

ANEMOUSITE IN ESSEXITE[1]

BY F. FITZ OSBORNE[2]

Abstract

Anemousite has been found in some Monteregian essexites. This raises some questions regarding the distribution of anemousite and the nomenclature of essexite. The name essexite may well be retained despite the occurrence of anemousite in the rock.

Introduction

The undersaturated feldspar, anemousite, of the Linosa basalts was described by Washington and Wright (10) in 1910. Nevertheless, undersaturated feldspars have not been described from many localities: Bancroft and Howard (3, pp. 22-24) have given an analysis of such feldspar from Mount Royal, and Barth (4, 5) has given the name pacificite to anemousite basalt.

The author has recently studied some of the Monteregian essexites, particularly those of Mount Johnson, and has found that undersaturated feldspar is abundant in some of them, and in some, associated with nepheline.

Anemousite from Mount Royal

There are only two trustworthy analyses of feldspar separated from Monteregian rocks and one of these, by M. F. Connor, formerly analyst for the Geological Survey of Canada, shows the presence of an undersaturated molecule in the feldspar. The analysis was published by Bancroft and Howard (3). The essexite specimen from which the feldspar was separated is from station 204+85 of the railway tunnel passing through Mount Royal. The locality is about 1000 ft. north of the south contact of the main mass of essexite with the Trenton limestone and is about 0.5 mile north of the campus of McGill University.

TABLE I
ANALYSIS OF ESSEXITE FROM MOUNT ROYAL

SiO_2	41.55%
TiO_2	3.92
Al_2O_3	14.84
Fe_2O_3	6.62
FeO	8.24
MgO	7.83
CaO	14.64
Na_2O	1.93
K_2O	0.25
H_2O	0.19
MnO	0.15
P_2O_5	0.10
CO_2	0.19
Cl	Trace
Cr_2O_3	None
SO_3	None
S	0.16
SrO	None
BaO	None
NiO	Trace
CoO	Trace
Total	100.61
Less O for S.	0.05
	100.56

The rock is a rather abnormal facies of the essexite in which a well marked ophitic texture is developed; olivine is included poikilitically in augite which is rimmed with alkaline amphibole, and both augite and amphibole are moulded on laths of plagioclase. A mode for the rock has been given (3), but it does not agree with the norm of the analyzed specimen in that the mode gives too high a proportion of feldspar. The analysis of the rock by J. B. Robertson is shown in Table I.

[1] *Manuscript received February 5, 1935.*
Contribution from the Department of Geology, McGill University, Montreal, Quebec, Canada.
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Bancroft and Howard recognized only one feldspar in this rock, but the writer has been able to distinguish two in their thin sections. One is a normal plagioclase zoned from about An60 to An82 in the core. In one thin section there is less of this plagioclase than anemousite, but in another there is more. The two feldspars differ in several respects. The plagioclase tends to be idiomorphic and the anemousite to border it, but the latter is itself somewhat idiomorphic toward amphibole and pyroxene. The indices of the more calcic zones of the plagioclase are noticeably higher than those of the anemousite. The plagioclase is twinned after the albite and Carlsbad laws with very subordinate pericline and Baveno twinning. The anemousite shows some albite twinning, but the prominent twinning is according to another law: this appears to be pericline with a composition face near 100. Sections of anemousite simultaneously perpendicular to 010 and 001 are nearly parallel to the composition face of the twins, and show the positive acute bisectrix emerging at about 20° from the centre of the field. Corresponding sections of the plagioclase show eccentric emergence of the bisectrix α. Boundaries of lamellas of polysynthetic twinning in the anemousite lack sharpness, and composition faces of the twins are irregular. The plagioclase lamellas in all sections except those much inclined to the composition face are sharply defined and straight.

The optic-axis interference figures of the anemousite show isogyres colored red on the concave side and the dispersion is thus $\rho < v$, whereas the corresponding isogyres of plagioclase are black. The dispersion is so strong that extinction is incomplete in white light, and the section changes from blue to brown near the extinction position. The anemousite is positive and the optic-axial-angle is about 60°. The indices of refraction determined on an uncovered thin section using freshly standardized oils are $\alpha = 1.559$, $\beta = 1.562$, $\gamma = 1.566$.

Connor's analysis of the undersaturated feldspar and the molecular proportions after deductions for iron ore minerals, diopside and olivine are shown in Table II.

TABLE II

ANALYSIS OF UNDERSATURATED FELDSPAR

	Connor's analysis	Molecular proportions after deductions
SiO_2	49.06	.8086
TiO_2	0.14	
Al_2O_3	30.96	.3049
Fe_2O_3	0.55	
MgO	0.15	
CaO	13.05	.2292
Na_2O	4.79	.0773
K_2O	0.50	.0053
MnO	0.04	
Loss on ignition	0.76	

The recalculated analysis is, by weight, Or 3.00, Ab 21.81, An 64.72, Ne 10.48%, but, as shown before, the feldspar is probably a mixture of about 50% of anemousite and 50% of calcic plagioclase. If the composition of the plagioclase be estimated as 75 weight per cent anorthite, the composition of the anemousite is Or 6.00, Ab 18.62, An 54.44, Ne 20.96%. The assumption on which the recalculation is based may be in error in several particulars. The plagioclase itself may be slightly undersaturated and it may

contain some potash. However, it appears to be definitely earlier than the anemousite and is of normal optical character. In any case the analysis proves the presence of an undersaturated feldspar.

Bancroft and Howard give a specific gravity of 2.705 for the mixture. If the specific gravity of the anemousite is calculated assuming 50% plagioclase of specific gravity 2.72, the result is 2.68 which is in good agreement with the feldspar from Linosa.

Mount Johnson

If the mass of plutonic melilite rock cutting the pre-Cambrian near Oka be excluded, Mount Johnson is the smallest of the Monteregian hills in outcrop area. Nevertheless, it is one of the most interesting, for five facies of plutonic rocks arranged as vertical, hollow, coaxial cylinders may be recognized. The core is of olivine essexite, surrounded by a fine-grained essexite known in the monument-stone trade as Ebony. The next facies is also an essexite which, on account of the pellucid character of its feldspars, resembles the stone from Quincy and is known commercially as Canadian Quincy. These are surrounded by a porphyritic rock for which N. L. Wilson and the writer have coined the term monnoirite. The outer annulus is a pulaskite porphyry grading to a pulaskite near the hornfels collar that surrounds the mountain. The rock of the core is the finest grained but the granularity does not increase uniformly outward. The coarsest appears near the contact of monnoirite and pulaskite porphyry. The feldspar of the several facies at Mount Johnson shows considerable differences in composition. It was the anomalous optical properties of the anemousite in the olivine essexite that led to the critical examination of the composition of the feldspar. The anemousite is more abundant in the core of the mountain than in the coarser facies, but it is found also in the Ebony and the Canadian Quincy. The monnoirite and the pulaskite are almost saturated rocks, and the feldspar they contain appears to be of normal composition. An analysis of the feldspar from the pulaskite shows no deficiency of silica, and the feldspar of the monnoirite is of the rhomben porphyry type.

Olivine essexite from the core of the mountain is millimetre grained, with platy phenocrysts of plagioclase as much as 12 mm. long. Augite occurs in two generations: the larger crystals are as much as 9 mm. long and 2.75 mm. wide, but the smaller ones are nearly equidimensional and are 0.12–0.42 mm. in diameter. The olivine is 0.1–0.2 mm. in diameter and the opaque minerals, which form 8% of the rock, are less than 0.25 mm. in diameter. Most of the feldspar and nepheline is from 0.5 to 2 mm. in diameter. The larger crystals of pyroxene and the phenocrysts of plagioclase partake of the vertical fluidal arrangement of the constituents which is a conspicuous feature of most of the plutonic facies on the mountain.

Pyroxene is of two kinds separable in favorable cases by optical properties. Part of it is a normal titaniferous augite showing strong dispersion and having an optic-axial-angle near 60°. The other is recognizable among the

smaller grains of the first generation of pyroxene. It has no appreciable dispersion and lacks the hour-glass structure seen in the augite. The optic-axial-angle determined by Mallard's method with a microscope, calibrated by means of oriented plates of minerals of known angle and indices, and checked with an Abbe apertometer, is between 50 and 52°. This pyroxene may be rimmed by the alkaline hornblende, but such is not the case for the normal augite. The alkaline hornblende and biotite are in an ophitic relation to the feldspar and are apparently of late development. The anemousite rock from Mount Royal is markedly ophitic. Nepheline appears to have crystallized only slightly later than the bulk of the anemousite.

Apatite occurs in large crystals and in fine needles cutting silicates.

The principal interest in the rock centres on the feldspar. Adams (1, p. 258), in describing the petrography of Mount Johnson, mentions the occurrence of pyroxene, biotite, amphibole, plagioclase, potash feldspar, nepheline and accessories. Examination of Adams' thin sections fails to show any potash feldspar. The thin sections were cut about 30 years ago, and the balsam has the relatively high index of 1.545, but careful search of the thin sections failed to disclose any abundant mineral with indices less than that of the Canada balsam, proving the absence of potash feldspar.

The plagioclase phenocrysts show sharply defined albite lamellation with Carlsbad twinning and rarely Baveno and pericline twinning, the latter with the normal composition face. The cores of the crystals are as calcic as An40, but the outer parts are considerably more sodic than this, appearing on some crystals to grade outward to An20. In places anemousite appears to rim plagioclase as if it were in reaction relation to it, but most of the anemousite is alone or in a degenerate sort of coarse intergrowth with nepheline.

The anemousite is triclinic in crystallization. Albite twinning is rare and the lamellas are faint. The common twinning is apparently pericline and the composition face is near the front pinacoid, for sections showing two cleavages nearly at right angles show no twinning—presumably because they are nearly parallel to the composition face. Such faces show nearly central emergence of γ and the optic axis near the edge of the field. The optic-axial-angle, as determined by Mallard's method, is between 60 and 65°. The sign is positive. In a few places the anemousite appears to have unmixed in two feldspars with the components in lenses approximately parallel to 010. In most, if not all, orientations the junction lines of adjacent twins are blurred and far from straight. In many places they actually show re-entrant angles. The dispersion is greater than that of plagioclase but less than that of the anemousite from Mount Royal and is $\rho < v$. Of all the properties, the irregularity of the twinning lamellation and the optic-axial-angle show the greatest constancy for the anemousite, for a variation in composition is indicated by range of extinction angle (on 010, 10–30°), indices and density.

An unsuccessful attempt was made to separate the anemousite from the plagioclase and other rock-forming minerals by the use of heavy liquids. The variation in the properties came to light in the course of this work. It was possible to separate the more calcic plagioclase from anemousite, but the other fractions all showed some of this mineral, and the anemousite was distributed through several fractions. Part of the anemousite sank immediately in a solution of density 2.672, another fraction sank in four hours, yet another remained suspended in the liquid for 20 hr., and still another floated on it. In general, the lighter fractions have the lower indices of refraction. The indices for one composition ($d = 2.672$) determined by immersion are $\alpha = 1.559$, $\beta = 1.562$ and $\gamma = 1.566$.

Despite the fact that it was not possible to make a separation of the anemousite for analysis, it is possible to arrive at the composition of the mineral indirectly. Adams has given the results of a Rosiwal analysis of a specimen which was analyzed by Connor. Adams states that the determination was based on several thin sections and more than 500 intercepts were made. He noted that the quantity of nepheline was lower than that demanded by the norm, but he was inclined to attribute that result to the imperfections of the Rosiwal method. It is probable that the Rosiwal determination is substantially accurate and the deviations are due to the presence of undersaturated feldspars. His determination of the amount of nepheline was checked by etching and staining.

A number of analyses of minerals occurring in the Monteregian rocks are also available, so it is possible to calculate the composition of the anemousite from the Rosiwal and chemical analyses of the rock with reasonable accuracy. This method yields only the average composition of the feldspar, and it is necessary to assume in the calculation that the plagioclase is saturated and contains little or no potash. The optical properties suggest that this assumption is justifiable.

For the calculation, an amount of apatite sufficient to account for all the phosphoric acid was determined and deducted and the percentages by weight

TABLE III

CALCULATION OF THE COMPOSITION OF THE ANEMOUSITE FROM MOUNT JOHNSON

	%	SiO_2	TiO_2	Al_2O_3	Fe_2O_3	FeO	MgO	CaO	Na_2O	K_2O	MnO
Nepheline	6.00	2.60		2.06				0.06	0.98	0.31	
Pyroxene	13.25	6.74	.10	.56	0.19	0.55	2.12	2.97			0.02
Amphibole	1.26	.49	.06	.15	.05	.15	.13	.16	.04	.02	.01
Biotite	3.97	1.31	.11	.41	.35	1.09	.03	.03	.04	.31	.11
Olivine	1.36	.52				.34	.58	.03			
Opaques	7.89		1.44		2.50	4.28					.02
Apatite	2.52							1.39			
Sum		11.66	2.71	3.18	3.09	6.41	2.86	4.64	1.06	.64	.15
Analysis		48.69	2.71	17.91	3.09	6.41	3.06	7.30	5.98	3.10	.15
Difference		37.03		14.73			.20	2.66	4.92	2.46	

were then recalculated to give the same total as the chemical analysis. The compositions of the minerals were selected as follows: nepheline, Brogger's average from ijolite; augite from Mount Royal; amphibole from Mount Johnson; biotite from Mount Royal; olivine from Mount Royal. The excess of MnO, Fe_2O_3, FeO, TiO_2 are allotted to opaques. The results of the calculation are shown in Table III.

The agreement between calculation and analysis thus obtained is very satisfactory: the small excess of MgO may be present as spinel in the opaques or as clinoenstatite in the pyroxene, in any case it is so small it may be disregarded. The silica, alumina, lime, soda and potash may be allotted to feldspar, which forms 62% of the rock. The bulk composition by weight of the feldspar is Or 14.57, Ab 26.83, An 13.22 and Ne 8.06%.

The writer estimates the amount of plagioclase as about one-third of the total feldspar, or 20% of the rock. On the average it contains about 30% by weight of anorthite. The approximate average composition of the anemousite is Or 34, Ab 30, An 17, Ne 19%, after deducting plagioclase. In this case, also, potash feldspar may be dissolved in the plagioclase, and thus the amount of Or in the above composition would be reduced. This mineral might be regarded as an anemousite-bearing potash oligoclase (7). This occurrence is of particular interest in that nepheline appears as a separate phase which crystallized simultaneously with anemousite.

Bowen and Greig (6, p. 211) have suggested that the presence of carnegieite (soda anorthite) may not be the best explanation of the undersaturation of these feldspars (see Ref. 2, pp. 326-334). It is worth noting in this connection that the analyses of the Mount Royal feldspar and the calculated feldspar show less alumina than is necessary to account for the bases.

The analyses and calculations presented in this paper appear to be sufficient evidence that an unsaturated triclinic feldspar exists in some essexites.

To sum up, the anemousite feldspars of the Monteregian province appear to have a higher dispersion than the normal plagioclase, they are optically positive and the angle between the optic axes is less than in any plagioclase. The twinning bands are less distinct than those of normal plagioclase, and a lamellar twinning approximately parallel to 100 is found. It is noteworthy that some of the potash oligoclases share some of these properties. A feldspar of the rhomben type occurring in the monnoirite at Mount Johnson has a higher dispersion than the normal plagioclase. In this respect it resembles the feldspar described by Quensel (9, p. 9) which is optically positive and has a notable dispersion. A feldspar from Mount Johnson occurring in an almost saturated pulaskite porphyry was analyzed by N. L. Wilson under the supervision of the writer. The powder was treated with concentrated hydrochloric acid in order to destroy the small amount of zeolitic minerals and sodalite. This feldspar shows a high dispersion similar to that of the Mount Johnson anemousites, but the analysis shows that the feldspar is saturated; therefore, the existence of a strong dispersion must be used with caution as a criterion for anemousite.

The compositions of anemousites are given in Table IV. Of these only one, *viz.*, the feldspar from Linosa, has been analyzed alone. The Mount Royal material was diluted with plagioclase, but inasmuch as it is definitely undersaturated it is of value in proving the existence of anemousite in a plutonic rock. The compositions given by Barth and the one from Mount Johnson are calculated from the analyses of the rocks, the presence of the anemousite type of feldspar being confirmed by optical properties. It is uncertain whether solutions of such diverse composition should be included under one name. Washington and Wright (10, p. 62) anticipated that an undersaturated series of feldspars might be found and new names given for compositions different from that of the Linosa feldspar. No easy method of division appears and, until analyses of pure material are available, anemousite may be used for the whole series.

TABLE IV

Compositions of anemousites
Weight per cent

	Barth pacificite							
Or	22	32	9	8	10	28	39	18
Ab	33	9	54	40	56	29	30	40
An	12	11	12	43	31	2	3	33
Ne	33	48	24	15	3	42	28	9

	Barth phonolite		Osborne Mt. Johnson	Osborne Mt. Royal	Washington and Wright Linosa
Or	14	36	34	6	4
Ab	44	55	30	19	36
An	11	7	17	54	54
Ne	31	5	19	21	6

TABLE V

Composition of 13 essexites and two olivine essexites from Mount Johnson

	I	II	III
SiO_2	48.98	48.69	48.41
TiO_2	1.99	2.71	2.70
Al_2O_3	17.35	17.91	18.51
Fe_2O_3	3.83	3.09	4.62
FeO	6.30	6.41	5.54
MnO	.20	.15	n.d.
MgO	3.52	3.06	3.00
CaO	7.54	7.30	7.79
Na_2O	5.54	5.95	5.66
K_2O	2.73	2.56	2.14
H_2O+	1.24	.95	.97
P_2O_5	.78	1.11	1.31
	100.00	99.97	100.75

I. Osann-Rosenbusch, average of 13 essexites.
II. Olivine essexite, N. N. Evans, analyst.
III. Olivine essexite, N. L. Wilson, analyst.

Anemousite Essexite

The finding of anemousite in the Monteregian essexites raises two questions. Is the mineral common in essexites, and does its presence make it necessary to give such a rock a new name? In answer to the first question, the mineral appears to be of fairly widespread occurrence in the Monteregian essexites and in some of the nepheline syenites. In the nepheline syenites it forms a rim around plagioclase, and is not commonly twinned. The twinning lamel-

lation in some anemousite is not noticeable and doubtless it is this fact, coupled with the considerable content of potash, that influenced some petrographers in mentioning potash feldspar as a constituent of the rock. The term essexite was proposed by Sears for the rock from Essex County, Mass., but he later decided that this rock has resulted through metasomatism of a gabbro by nepheline syenite, and he mentioned the rock from Mount Johnson as a typical example of a magmatic rock of that composition. Table V shows the average composition of 13 essexites and two analyses of olivine essexites from Mount Johnson. The analysis given in Column III has not been previously published. This would suggest that the name essexite would be retained, but Barth decided that it was advisable to give the new name *pacificite* to some lavas of composition of essexite because of the presence of anemousite.

Note

Since the above paper was written one by Ernst and Nieland (7) has appeared. They have analyzed some feldspar from Linosa megascopically similar to that analyzed by Washington and Wright, and found no undersaturation.

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VISCOSITY EFFECTS IN A CHANNEL OF SMALL EXPONENTIAL DIVERGENCE[1]

By G. N. Patterson[2]

Abstract

An experimental investigation of a flow form, deduced by Blasius from theoretical considerations, was carried out with air as the medium. A photographic method of measuring velocity distributions was adopted, and a diverging channel was designed from considerations based on the theoretical treatment and on requirements arising out of the experimental method. At a Reynolds number of 35, curves of velocity distribution were measured at various positions along the channel, and comparisons were then made with the corresponding theoretical curves. Good agreement was found over the region of the channel to which the theoretical results could be applied. A study of the experimental curves in that part of the channel to which the theoretical results could not be applied quantitatively showed further that the general flow characteristics described by Blasius are to be found in this region.

Introduction

Viscosity effects in air moving near a curved surface can be conveniently tudied by observing the flow of air through diverging channels. The roblem of the flow of a viscous incompressible fluid through diverging hannels has been treated theoretically by Blasius (1), who obtained an pproximate solution for the case of a very gradual divergence. Since the notion of air at very low velocities may be regarded as essentially that of a iscous incompressible fluid, it was considered that the flow characteristics lescribed by Blasius could be shown to exist in slowly moving air. As described y the author in the first paper on this subject (3), the Blasius flow form is o be found in the range $25 \leq R \leq 36$ of the Reynolds number. In order o investigate more thoroughly these viscosity effects near a curved surface, he present work was undertaken to obtain a quantitative comparison etween the curves of velocity distribution deduced by Blasius and those btained by direct measurement.

Summary of Blasius' Work

Blasius considered the two-dimensional flow of a viscous incompressible luid through a channel which diverges in a gradual symmetrical manner ccording to a relation of the general form

$$z = f(\epsilon x), \tag{1}$$

here (x, z) is a point on the wall of the channel, and ϵ is a small quantity. he choice of the particular form of Equation (1) was made subject to two onditions:—

(1) The first derivative of z with respect to x must be small.

(2) Each differentiation with respect to x must decrease the order of agnitude by ϵ.

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[2] *Holder of a bursary under the National Research Council of Canada.*

By a method of successive approximations, arising out of a treatment of the orders of magnitude of the terms in the hydrodynamical equations of motion, Blasius showed that, as a first approximation, the curve of velocity distribution along any line perpendicular to the axis of the channel was a parabola of the form

$$\frac{u}{\bar{u}} = \frac{3}{4}\left(\frac{w}{z}\right)\left\{1 - \left(\frac{y}{z}\right)^2\right\}, \tag{2}$$

where u is the component of velocity in the direction of x at any point (x, y) in the fluid, $\bar{u}$ is the mean velocity at $x = 0$, and w is the width of the channel at $x = 0$. In order to obtain a better solution, Blasius then carried out a second approximation which introduced a correction factor, and Equation (2) became

$$\frac{u}{\bar{u}} = \frac{3}{4}\left(\frac{w}{z}\right)\left\{\left[1 - \left(\frac{y}{z}\right)^2\right] + \frac{3}{4} R \frac{dz}{dx}\left[\frac{1}{42} - \frac{11}{70}\left(\frac{y}{z}\right)^2 + \frac{1}{6}\left(\frac{y}{z}\right)^4 - \frac{1}{30}\left(\frac{y}{z}\right)^6\right]\right\}, \tag{3}$$

where R is the Reynolds number (3, p. 778, footnote). An investigation of this equation revealed the existence of two points, symmetrically situated on each wall of the channel, at which the flow leaves the wall. Beyond each point of "break-away" is a region of reversed flow in which the particles near the walls move in the negative direction of the axis of x.

Blasius used the relation

$$\left(\frac{\partial u}{\partial y}\right)_{y=z} = 0 \tag{4}$$

as the condition for the break-away. Substituting from Equation (3) he obtained

$$R\frac{dz}{dx} = \frac{35}{2} \tag{5}$$

as the equation from which may be deduced the value of x at which the break-away occurs.

Blasius also obtained an equation for the component of transverse velocity, v. Since v is of the order of $\frac{dz}{dx}$, and therefore very small compared with the axial velocity, u, it does not play an important part in an investigation of the motion of the fluid.

Method of Experimental Investigation

It was decided to investigate the photographic method of Nisi and Porter (2), which could be used for the measurement of low velocities. The method is similar to that used in ultramicroscopy. The smoke generated by burning magnesium ribbon is mixed with the air, and the magnesia particles, which are illuminated by a horizontal sheet of light, are observed on the focusing screen of a camera fitted with a microscope objective. By replacing the screen with a photographic plate, the motions of the particles can be recorded, and curves of velocity distribution can be obtained from the lengths of the particle tracks made during a known time of exposure.

From the standpoint of the present work certain modifications of this method are required. The regions investigated by Nisi and Porter were the wakes behind bodies of various shapes where the velocity is low compared with the velocity of the undisturbed flow. However, in the case of a diverging channel, it is necessary to obtain photographs which show conditions at the same instant over an area extending from one wall to the other. Thus each photograph must include tracks which are made by particles moving in regions of both low and high velocity.

Preliminary experiments on the relation of the quality of the photograph to the speed of the particle showed that the velocity of the image of the particle on the photographic plate was a very important factor. These experiments indicated that photographs of rapidly moving particles could be obtained if the velocity with which the image crossed the photographic plate could be decreased sufficiently below the speed of the particle itself. This required a reduction, rather than a magnification, of the area to be photographed.

Further experiments were then carried out to determine the feasibility of obtaining velocity distribution curves by taking a reduced photograph of the flow and examining the negative under a microscope. After many trials it was found that the smoke particles were not sufficiently distinct to yield dependable results. The experiments showed that the success of the method depended upon the weight, size, and reflecting power of the particle used. Further investigation of different types of particles revealed that the best results could be obtained with magnesium oxide dust particles.

The details regarding the optical system, the photographic arrangement, and the method of obtaining a good mixture of air and particles are contained in the author's first paper on this subject (3).

Design of the Channel

The design of the channel depends upon the conditions specified in the theoretical problem and upon restrictions arising from the experimental method. The theoretical conditions, which the channel must satisfy, are:—

(1) The walls must be curved according to some particular form of Equation (1), subject to the conditions governing this choice.

(2) The dimensions of the channel are to be such that a good two-dimensional flow is assured.

(3) If the value of $\frac{dz}{dx}$ at $x = 0$ is made sufficiently small so that it may be neglected, then the velocity distribution at $x = 0$ is parabolic. The restrictions placed on the dimensions of the channel by the experimental method are:—

(1) The height of the channel is limited by the relatively short distance between the camera lens and the central horizontal plane of the channel which is necessary in order to obtain good photographs.

(2) In the narrowest part of the channel (at $x = 0$) the ratio of the height of the channel to its width must be such as to allow the camera lens to collect sufficient light to produce a good photograph.

(3) For all values of x the distance between the walls is limited by the requirement that the image of both walls must appear on the negative.

The particular form of Equation (1) which Blasius suggests is

$$z = a + be^{\epsilon x}, \tag{6}$$

where a, b, and ϵ are constants to be chosen. At $x = 0$

$$a + b = \tfrac{1}{2}w. \tag{7}$$

Further

$$\left(\frac{dz}{dx}\right)_{x=0} = \epsilon b, \tag{8}$$

and hence ϵb must be small in order that a parabolic distribution of velocity may exist at $x = 0$. If $a = 0$, Equation (6) becomes

$$z = \tfrac{1}{2}we^{\epsilon x}. \tag{9}$$

Now, by the second of the theoretical conditions given above, it is necessary to choose w as small as possible, since the height of the channel is already limited by the first of the experimental restrictions. On the other hand, w must be large enough to satisfy the second experimental restriction. Investigations showed that the best conditions were obtained by putting w equal to 0.5 cm., making the ratio of the height of the channel to its width 16.

The choice of ϵ is more difficult to make since the restrictions on it are of a more general nature. According to Equation (8) the smaller ϵ is, the more nearly does Equation (3) approximate to Equation (2) at $x = 0$. On the other hand, the third experimental restriction imposes a second limit on ϵ. Equation (3) shows that the deviation of the flow conditions from the parabolic at any position in the channel ($x > 0$) depends upon the product $R\frac{dz}{dx}$. For any particular line (x = constant) in the channel we may write

$$R\frac{dz}{dx} = R\epsilon z = k, \tag{10}$$

where k is a constant. Consider two channels made according to two different forms of Equation (9) obtained by selecting two values of ϵ. The flow conditions along a line in the first channel will be similar to those along a line in the second channel if the product ϵz is the same for both lines, the value of R being maintained constant. Thus, as smaller values of ϵ are considered, similar flow conditions will be found to exist at correspondingly larger values of z.

This fact was verified by experiment. Tests were made with two channels for which $\epsilon = 0.1$ and $\epsilon = 0.3$, and it was found that, for the same Reynolds number, the break-away occurred at the larger value of z in the channel for which $\epsilon = 0.1$. Therefore, in order to obtain photographs in the region of the break-away, it is necessary to choose ϵ so that z is not too large.

After making a series of experimental tests it was finally decided that $\epsilon = 0.1$ gave the best compromise between the experimental and theoretical requirements. Equation (9) therefore becomes

$$z = 0.25e^{0 \cdot 1x}, \tag{11}$$

which satisfies the first theoretical condition. Calculations based on this value of ϵ show that the error involved by using Equation (2), when $x = 0$, instead of Equation (3) is about 1%, which is about the same order as the error in the measurements of the particle paths.

In order to produce a parabolic distribution at $x = 0$, the walls of the channel for $x < 0$ were made straight and parallel, with a distance w between them. The value of $\dfrac{dz}{dx}$ at $x = 0$ was so small that the two parts of the channel could be joined at $x = 0$ without introducing any discontinuity. For the construction and dimensions of the channel and the experimental arrangement for producing a steady flow, the reader is referred to the author's first paper (3).

Experimental Technique

Measurements of the velocity distributions throughout the channel were carried out, at $R = 35$, where the best conditions were considered to prevail. The camera and the optical system were mounted on the same base, so that they could be moved to different points along the channel without disturbing the relative positions of the camera and the horizontal sheet of light. The steadiness of the flow was tested by taking a series of photographs at different times in the region about the line $x = 0$. Curves of velocity distribution along the line $x = 0$ were obtained from measurements of the photographs, and the mean velocity for each curve was calculated. The maximum variation was found to be less than 1%. Photographs of the particles were then taken at various distances along the channel ($x > 0$), the mean velocity being kept constant at 10.5 cm. per sec. Lines corresponding to values of x were chosen by inspection of the photographs, and the velocity distribution along each was measured. The values of $R\dfrac{dz}{dx}$ for these lines were calculated and found to be 1.46, 2.44, 3.87, 6.00 and 8.70. Beyond the value 8.70, measurements were not possible since the value of z was too great for both walls of the channel to show on the negative.

By reducing the curve for Equation (11) to the scale of the photographs and then fitting the negative to the curve, the axial line on each negative was found. The photograph could then be orientated with respect to the cross hairs of a traveling microscope. The axial line could be found very accurately by observing the positions of particles adhering to the walls. The equipment and methods used to measure the lengths of the particle tracks and the times of exposure are described in the first paper (3).

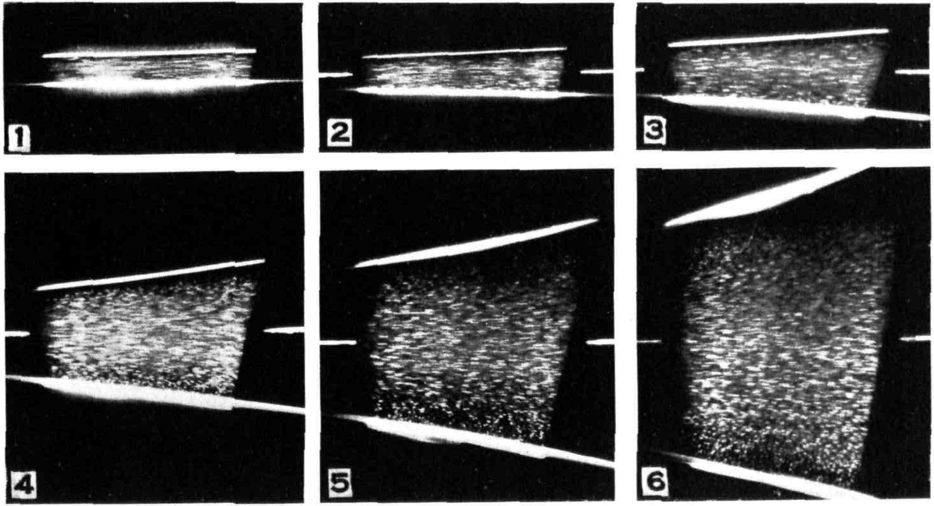

FIG. 1. *Particle tracks in the region of $x = 0$; time of exposure, 0.0166 sec.* FIGS. 2–6. *Particle tracks in regions corresponding respectively to the following values of $R\frac{dz}{dx}$: 1.46, 2.44, 3.87, 6.00 and 8.70; the times of exposure in order are 0.0166, 0.0273, 0.0393, 0.0519 and 0.0675 sec*

Results

In Figs. 1–6 are shown enlargements obtained from the negatives from which measurements of the velocity distributions were made. Successive photographs represent the flow conditions at positions along the channel corresponding to increasing values of $R\frac{dz}{dx}$. In each case the size of the image on the negative was 0.370 times the actual dimensions. The scale of the photographs may be judged from the fact that the line joining the centre points of the two walls in Fig. 1 is the line $x = 0$ at which the width of the channel is w (= 0.5 cm.). The times of exposure are longer as the width of the channel increases, that is, as the velocity decreases.

Although much of the detail observed on the negative under a microscope is lost in these enlargements, they will serve to show the general characteristics of the flow. It will be seen that there is a continuous reduction of the mean velocity as the channel diverges. The gradual formation of the low-velocity region near the walls, as the divergence increases, can be traced through the photographs. Along any line drawn perpendicular to the axis of the channel, it will be seen that the velocity rises from zero at the walls to a maximum in the centre of the channel. It will be noticed that the motion is symmetrical about the axial line. Since the particle paths near the walls are very short, these photographs do not indicate the general nature of the motion in the neighborhood of the break-away. For an illustrated discussion of the motion of the particles in the reversed flow regions, the reader is referred to the first paper (3). The gradual slowing-up of the particles near the walls, which precedes the break-away, is quite noticeable in Figs. 1–6. Since the light is incident on the inside surface of one wall, some light is reflected back to the other wall. In the photographs, the line which indicates the position of the wall through which the beam passes first has superimposed on it a second longer line, which is caused by the reflection of the light. These two lines are distinguishable when the negative is viewed through a microscope, and particles which lie on the line caused by reflection can be measured. The rapid reduction of the intensity of the light with the divergence of the beam is responsible for the fact that the field on one side of the channel is brighter than on the other. This is especially noticeable in Figs. 5 and 6, where many of the very faint paths cannot be reproduced. For experimental reasons it was possible to use only the divergent part of the beam.

It was found that the components of the paths perpendicular to the axis of the channel were too small for accurate measurement. In regions of the channel where the paths were long, the inclination to the axis of the channel was small, and in regions of large inclination the paths were short. However, in order to test the symmetry of the flow, the inclinations of the paths to the cross hair of the microscope were observed. It was found that paths on the axis of the channel showed no inclinations, and that the inclinations then increased uniformly as each wall was approached.

The curves of the velocity distribution corresponding to the selected values of $R\frac{dz}{dx}$ are shown in Fig. 7. For the purpose of making a comparison, the theoretical curves given by Equation (3) are also plotted. The results show that a parabolic distribution exists at $x = 0$, and that, for $R\frac{dz}{dx} = 1.46$, theory and experiment agree, for in both cases the theoretical and experimental curves are superposed. At $R\frac{dz}{dx} = 2.44$ the theoretical and experimental results show a divergence which increases as $R\frac{dz}{dx}$ increases. The curve at $R\frac{dz}{dx} = 6$ indicates that this is the position of the break-away, and at the final position, $R\frac{dz}{dx} = 8.70$, the curve indicates the existence of a *reversed flow. It should be noticed that the motion is two dimensional,* since the areas under the experimental curves are equal to the areas under the corresponding theoretical curves.

Discussion of Results

In the exponential type of channel, $\frac{dz}{dx}$ increases with increasing values of x. As $\frac{dz}{dx}$ becomes larger, the approximate theoretical results, deduced on the assumption that $\frac{dz}{dx}$ is small, will become less and less accurate until values of $\frac{dz}{dx}$ are reached for which Equation (3) is no longer an approximate solution. Whether or not the theory can be expected to hold up to and beyond the points of break-away depends upon the value of $\frac{dz}{dx}$ at which the flow leaves the walls. Equation (5) shows that, unless the type of viscous flow described by Blasius occurs at Reynolds numbers of the order of 200, the condition that $\frac{dz}{dx}$ will be small up to and beyond the break-away cannot be satisfied. Thus the range in which Blasius' results may be applied depends upon the value of R. For values of R lying in the range $25 \leq R \leq 36$ it is clear that Equation (5) cannot hold, and that the approximate results of Blasius cannot be considered to hold beyond about the value

$$R\frac{dz}{dx} = 2. \qquad (12)$$

The experimental results agree with this value. It can therefore be concluded that theory and experiment agree over the range of the quantity $R\frac{dz}{dx}$

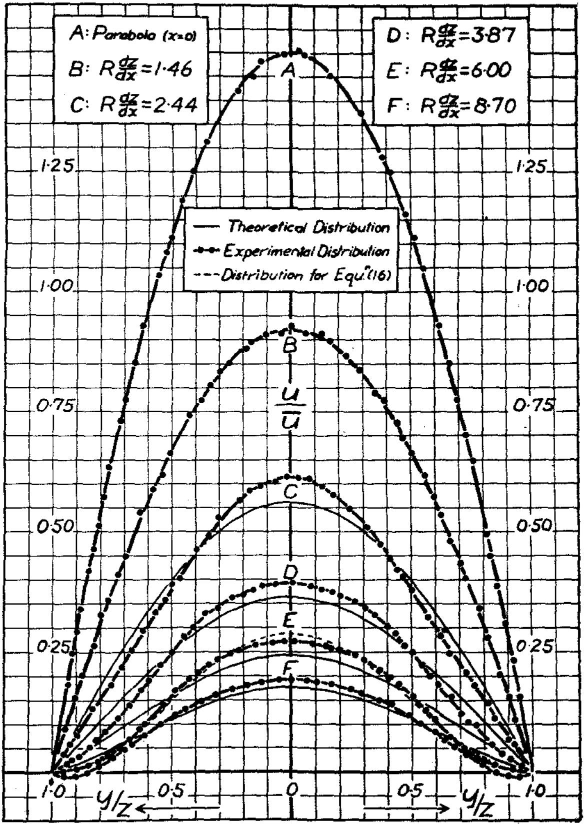

FIG. 7. *Theoretical and experimental curves of velocity distribution at various positions along the channel, showing the region of agreement between theory and experiment and the existence of a flow having the general characteristics of the type derived by Blasius.*

in which Blasius' results may be considered to hold when the Reynolds number is 35.

Although the theoretical results cannot be applied quantitatively beyond the line given by Equation (12), yet a comparison of the experimental curves in Fig. 7 with the theoretical curves shown by Blasius (1) indicates that the essential characteristics of the flow, deduced theoretically for regions of the channel in which $\frac{dz}{dx}$ is small, are also to be found in regions where $\frac{dz}{dx}$ is relatively large. It is possible, therefore, that a more general form of Equation (3) will hold in those regions for which $R\frac{dz}{dx} > 2$. If Equation (3) is written in the form

$$\frac{u}{\bar{u}} = \frac{3}{4}\left(\frac{w}{z}\right)\left\{\left[1 - \left(\frac{y}{z}\right)^2\right] + \beta R\frac{dz}{dx}\left[\frac{1}{42} - \frac{11}{70}\left(\frac{y}{z}\right)^2 + \frac{1}{6}\left(\frac{y}{z}\right)^4 - \frac{1}{30}\left(\frac{y}{z}\right)^6\right]\right\} \quad (13)$$

where β replaces the value $\frac{3}{4}$ in Equation (3), then according to Equation (4), the break-away will occur at

$$R\frac{dz}{dx} = 6, \quad (14)$$

if the constant has the value

$$\beta = \frac{35}{16}. \quad (15)$$

Equation (13) now becomes

$$\frac{u}{\bar{u}} = \frac{3}{4}\left(\frac{w}{z}\right)\left\{\left[1 - \left(\frac{y}{z}\right)^2\right] + \frac{35}{16} R\frac{dz}{dx}\left[\frac{1}{42} - \frac{11}{70}\left(\frac{y}{z}\right)^2 + \frac{1}{6}\left(\frac{y}{z}\right)^4 - \frac{1}{30}\left(\frac{y}{z}\right)^6\right]\right\}. \quad (16)$$

This equation is plotted in Fig. 7 and a comparison with the experimental curve for $R\frac{dz}{dx} = 6$ shows that the agreement is much better than that given by Equation (3). Hence, over the range $2 \leq R\frac{dz}{dx} \leq 6$ the value of β has risen from $\frac{3}{4}$ to $\frac{35}{16}$. Therefore β depends upon $R\frac{dz}{dx}$ and we may write

$$\beta = f\left(R\frac{dz}{dx}\right). \quad (17)$$

A further consideration of the curve for Equation (16) indicates that by adding higher powers of $\left(\frac{y}{z}\right)$ to the correction term, the shape of this curve can be made to correspond more closely to the shape of the experimental curve. These considerations lead to the conclusion that the generalized form of Equation (3) which would hold for $R\frac{dz}{dx} > 2$ is

$$\frac{u}{\bar{u}} = \frac{3}{4}\left(\frac{w}{z}\right)\left\{\left[1 - \left(\frac{y}{z}\right)^2\right] + F\left(R\frac{dz}{dx}\right)\Sigma\, a_n\left(\frac{y}{z}\right)^n\right\}, \quad (18)$$

where $n = 0, 2, 4, \ldots\ldots$, and the maximum value of n is greater than 6.

Acknowledgments

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MEASUREMENT OF THE VELOCITY OF SOUND IN LOW TEMPERATURE LIQUIDS AT ULTRASONIC FREQUENCIES[1]

BY ARNOLD PITT[2] AND W. J. JACKSON[3]

Abstract

An ultrasonic interferometer apparatus has been developed for the measurement of the velocity of sound in low temperature liquids. The method follows in general the conventional manner of producing sound waves in a liquid, *i.e.*, by the vibrations of a piezoelectric quartz plate driven at a high frequency. The velocity of sound in liquid oxygen and hydrogen at an ultrasonic frequency of 427 kilocycles per sec. was found to be:—oxygen, 912 metres per sec. (temp., −182.9° C.); hydrogen, 1127 metres per sec. (temp., −252.7° C.).

For the purpose of determining experimentally the velocity of sound in low temperature liquids, from which values of their compressibilities may be derived, a method of setting up ultrasonic vibrations in the liquid by means of a piezoelectric quartz plate was employed. Owing to the extremely low temperatures of the liquids and the relatively small quantities available, it was necessary to modify considerably the design of apparatus employed by previous workers (1,2).

Work on the velocity of sound in gases at low temperatures has been carried on at Leiden by Keesom (3,4,5), Itterbeek (3,4,5) and others. They employed an audible frequency resonance method and made accurate determinations of velocity, using a resonator chamber of fixed dimensions and varying the frequency of the sound wave to obtain resonance. By measuring the velocity of sound in dry air at 0° C. they were able to establish the accuracy of the method. Owing to the long sound wave-length relative to the size of the gas chamber resonator, correction factors were applied to take care of the effects of the wall and the end openings on the measured value of wave-length.

In selecting a method of measurement of velocity of sound in low temperature liquids, it was expected that the greatest difficulty would be met in avoiding boiling of the liquids, owing to the presence in the liquid of a metal column, and to the energy of the sound waves set up. Mechanical rigidity had therefore to be sacrificed in favor of low thermal conduction, and a resonance detector of unusual sensitivity developed so that the sound wave energy might be kept low. From work by Boyle (1) and co-workers, it was shown that at frequencies greater than 100 kc. per sec., with a liquid column of sufficient size, the relation between velocity and frequency was approximately constant.

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Contribution from the McLennan Laboratory, Department of Physics, University of Toronto, Toronto, Canada.

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Fig. 1 is a diagram of the sonic interferometer. The quartz-crystal disc and the reflector plate, R, were plane parallel with each other for all positions of the reflector. By means of the micrometer head, A, the reflector, R, could be moved up or down and readings of its position taken within 0.01 mm. Thermal loss was kept low by using very thin german silver tubing to support the mechanism in the liquid. The method of mounting the quartz plate presented the greatest difficulty of construction. Previous investigators (2, 7), working with liquids at ordinary temperatures, were able to enclose the crystal in a liquid-tight box and transmit the vibrations from the crystal to the liquid through a very thin metal diaphragm. This was accomplished by putting a drop of oil between the crystal and the diaphragm to secure intimate contact. Since this was impossible at low temperatures, a method of clamping the crystal, similar to that of Lack (6) was used. A film of platinum was sputtered over the faces of the crystal and it was mounted between the electrodes E_1 and E_2, being held very near the edges at the nodal region. This secured contact with the platinum films, the lower electrode E_2 being pressed against the crystal by means of the spring S_2. The crystal, clamp and spring were enclosed by a fibre shell F, and electrically shielded by a case H which was fastened to the main support tube G. Electrical connections to the electrodes were made by means of the tube G to which E_1 was fastened, and the lead T which ran through the shielding tube G to E_2. The whole construction was devised to slip into a Dewar flask as shown in the diagram. The dimensions of the Dewar flask were approximately 43 mm. inside diameter and 1 mm. wall thickness.

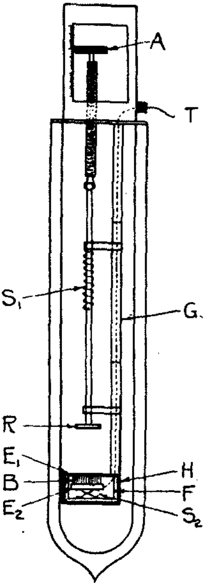

FIG. 1. *Sonic interferometer.*

The electrical wiring circuit for the ultrasonic generator is shown in Fig. 2. Section 1 of this circuit shows a crystal-controlled generator of conventional design, having a generated frequency of 427 kc. per sec. This was coupled to an intermediate screen-grid amplifier stage, which offered a high impedance path to any coupling back of energy from the output to the input stages. Section 3 shows a pentode power amplifier, having in the plate circuit a load impedance which could be adjusted by means of the condenser C_1. Radio-frequency voltage developed across this impedance was carried through C_2 and C_3 to the electrode marked E_2 in Fig. 1. E_1 was at ground potential and connected to the shielding.

To detect the condition of resonance in the interferometer system, a vacuum tube voltmeter circuit employing a sensitive detector type of tube was set up. Connection from the grid of this tube was made between C_2 and C_3. C_2 kept the d-c. potential at the plate of the power tube from being impressed

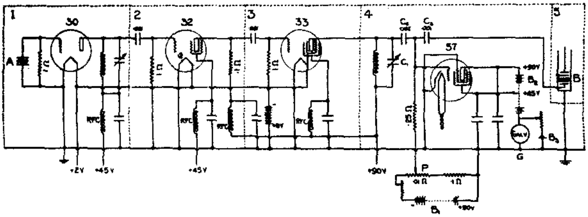

FIG. 2. *Electrical wiring circuit for the ultrasonic generator.* *(Ω = megohm; by-pass condensers are 0.1 μf.*

on the grid of the detector, and C_3 prevented the large d-c. biasing potential of B_1 from existing across the crystal and causing conduction effects in the liquids. Readings of plate-current variation were taken by means of a galvanometer having the steady value of d-c. current balanced out by means of the current from B_3. The high radio-frequency potential, which was continuously impressed at the grid of the detector, produced a large increase in plate current owing to the rectifying action of the tube. Voltage from the battery B_1 was taken from the potentiometer P to reduce the plate current and keep it at a value for which the rectifying action was a maximum.

The measurement of the wave-length of sound in a liquid in which the interferometer is immersed merely involves rotating the micrometer head A, and observing on the galvanometer in the detector circuit the periodic changes in plate current which occur as the column length between reflector and sound source is made an integral number of half wave-length distances. Vibrations in the liquid column are then in resonance with those of the crystal, with a resultant maximum amplitude in the crystal. Under this resonance condition, a sharp change in the piezoelectric voltage of the crystal occurs which may readily be detected. Knowing the half wave-length distance from the reflector displacement, and the driving frequency, the velocity in the liquid may be directly determined.

Preliminary measurements were made using liquids for which the velocity of sound is known, with a view to determining any error which might exist in the apparatus due to change in length of liquid column, effects of the walls of the Dewar flask, or other anomalous resonance effects which might be present. Owing to the exposed electrodes, only liquids of very low conductivity could be used. The sensitivity of the method was not great enough to make a determination in air at atmospheric pressure, owing largely to the method of clamping the crystal.

Having found by experiment that the piezoelectric effect was still in existence for quartz at liquid air temperatures, the interferometer was carefully immersed in liquid air and very sharp maxima were obtained. Boiling of the liquid air could not be observed for any variation in setting of the reflector, or when the oscillations of the crystal were started or stopped.

Attempts to measure the velocity of sound in liquid hydrogen, near the boiling point, were partly successful. Fairly good maxima were obtained, but some boiling was constantly going on and the presence of occluded gas in the liquid makes the result for the velocity uncertain. On manipulating the liquid hydrogen to prevent boiling, other gases were unavoidably admitted to the system. These froze and were deposited over the face of the crystal, thus preventing further measurements.

In liquid oxygen the determinations at the boiling point were very satisfactory. Exceedingly sharp maxima were obtained, and, although boiling took place at the liquid surface, conditions between the reflector and the crystal were very steady. Evaporation of the liquid oxygen to lower its temperature did not result in a sufficiently steady state for further measurements.

Table I shows results obtained for liquid oxygen and hydrogen at an ultrasonic frequency of 427 kilocycles per second. For comparison, values of velocity are given for the two elements in gaseous state and at different temperatures. All values in the table are those obtained at atmospheric pressure.

TABLE I

VELOCITY OF SOUND IN LIQUID OXYGEN AND HYDROGEN AT 427 KC. PER SEC.

—	Temp., °C.	Velocity, metres/sec.
Oxygen		
Gas	0	315.4
Gas	−182.9	177.6
Liquid	−182.9	912
Hydrogen		
Gas	0	1286
Gas	−252.9	357
Liquid	−252.7	1127

It is intended to do further work on the measurements of these and other liquids at low temperatures. It was observed in the case of liquid hydrogen that the reaction on the detector circuit at resonance was much less than was to be expected, unless a large decrease in piezoelectric effect should have occurred. This observation was corroborated by an attempt to measure the velocity of sound in liquid helium. No reaction effects could be observed even when the temperature of the liquid was well below the boiling point.

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INTERFEROMETER MEASUREMENTS OF THE HYPERFINE STRUCTURE OF SOME LINES OF SINGLY IONIZED BISMUTH[1]

By Stanley Smith[2] and J. S. Beggs[3]

Abstract

The hyperfine structure of nine lines of Bi II and one line of Bi III has been measured by means of a quartz Lummer plate and a glass Lummer plate used in conjunction with a Hilger E 1 spectrograph. The light source was a water-cooled hollow cathode discharge in helium. Of the lines investigated the hyperfine structure of some had already been resolved either totally or in part by Fisher and Goudsmit using a 21 ft. grating, but for the lines λ6808, 6600, 4272 and 4259 the hyperfine structure has been obtained for the first time. The separation factors for the terms $6p_{\frac{1}{2}}5f_{3\frac{1}{2}}14_4$ and $6p_{\frac{1}{2}}6d_{2\frac{1}{2}}8^\circ_3$ have been found.

The interaction constants of the $5f_{2\frac{1}{2}}$, $5f_{3\frac{1}{2}}$, and $6d_{2\frac{1}{2}}$ electrons have been calculated.

Introduction

The most important of the terms in the spectrum of singly ionized bismuth were discovered by McLennan, McLay and Crawford (6) who at the same time found a few of the terms to have large hyperfine structure. Fisher and Goudsmit (3), by means of a 21 ft. concave grating, have investigated the structures of a number of the terms. In a later paper by Crawford and McLay (1) the analysis of this spectrum was extended. In the present investigation, Lummer plate interferometers have been used to obtain the structure of some of the lines hitherto unresolved. In the investigation of the two lines λ6600 and 6808 it was necessary to use the Lummer plate because of the difficulty of getting sufficient intensity in higher orders in this region with a grating. Other lines not previously resolved but showing structure in the interferometer patterns which have also been studied are λ4259 and 4272. The lines λ5719, 5270, 5209, 5144 and 4392 and the line λ4561 of Bi III have been re-examined.

Experimental Procedure

The source was a water-cooled hollow-cathode discharge in helium at a pressure of about 4 mm. of mercury. The Pyrex glass tube, of length 30 cm. and internal diameter 4.6 cm., had an aluminium anode and a molybdenum cathode of length 7 cm. and diameter 1.7 cm. The discharge was excited by applying a potential of about 900 volts supplied by a d-c. dynamo, and the current through the tube was normally about 0.25 amp. The light from the cathode was concentrated on the reflecting prism of the Lummer plate by means of a quartz lens, and the fringes formed were focused on the slit of a Hilger E 1 spectrograph by a quartz-fluorite lens of focal length 23 cm. The

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plane of the Lummer plate was horizontal, *i.e.*, perpendicular to the slit o the spectrograph. In order to control the position of the source relative to the spectrograph and Lummer plate, the discharge tube and accessories, such as circulating pumps, purifying tubes and helium reservoirs, formed a complete unit rigidly attached to the top of a table provided with short legs of adjustable height which rested on another table. The latter traveled on wheels along a track permitting the maintenance of the alignment of the tube with the optical system. The Lummer plate and its adjustable holder (and the Nicol prism in the case of the quartz plate) were housed in a double-walled box made of "Ten-Test". In this were windows which were formed by the quartz condensing lens and the quartz-fluorite projecting lens, the latter being rigidly attached to the spectrograph. The interferometer system and the spectrograph stood on a rigid steel base plate. As some of the exposures, especially when the red region was photographed, were of 14 hr. it was necessary to take precautions to maintain the temperature of the spectrograph and the interferometer as constant as possible. For this purpose two mercury-contact thermostats were used. One of these kept the room temperature constant with a maximum variation of about 0.5° C. and the other maintained the temperature of the box constant, the amplitude of the variation of the air temperature in the box being about 0.1° C. The temperature changes in the Lummer plate itself would probably be less than this. A glass Lummer plate, of length 13 cm. and thickness 0.4872 cm., and a quartz plate, of length 13 cm. and thickness 0.4493 cm., were used. The optic axis of the quartz plate was parallel to the long edge of the plate. When this plate was used a Nicol prism with its short diagonal horizontal was inserted between the condensing lens and the plate so that the light traveled through the plate as ordinary rays. The Lummer plate was always adjusted to give the fringes from both the top and the bottom of the plate simultaneously. The adjustment of the angle of incidence of the light on the reflecting prism of the Lummer plate to obtain symmetry between the two sets of fringes was found to be very critical. For the red region of the spectrum Eastman Process Panchromatic and Spectroscopic S III plates were used, and for the green and violet regions Eastman 33 and Process plates were used. The fringes were measured on a comparator.

Method of Measurement

As is well known in the theory of the Lummer plate, a small increase in the wave number of the light shifts an interference fringe of a given order towards the centre of the pattern, *i.e.*, the angle of emergence of the light forming the interference fringe is decreased. An important factor in the computation of the wave number differences between the components of the hyperfine structure of a spectral line is the quantity $\Delta\nu$, the change in the wave number which would make a fringe of a given order due to light of wave number $\nu + \Delta\nu$ coincide exactly with the fringe of the next lowest order

formed by the light of wave number ν. $\Delta\nu$ was calculated from the equation

$$\Delta\nu = \frac{\sqrt{\mu^2 - 1}}{2t\left(\mu^2 - 1 + \lambda\mu\frac{d\mu}{d\lambda}\right)},$$

where t is the thickness of the plate in cm. and μ is the refractive index of the plate for light of wave-length λ. To determine μ and $\frac{d\mu}{d\lambda}$ a Cauchy formula was used in the case of the glass plate. For the quartz plate the dispersion formula given in Drude's Theory of Optics (2, p. 391) was used.

Having found the value of $\Delta\nu$ for a given complex spectral line, the wave number differences between the components were computed by the method already used by McLennan and McLeod (5).

If D_1, D_2, D_3, etc., represent distances between the corresponding fringes in the two halves of the pattern due to light of wave number ν, and D_1', D_2', ... represent the corresponding quantities for fringes due to light of wave number $\nu + d\nu$, then $d\nu$ is obtained from the equation

$$d\nu = \frac{P}{Q}\Delta\nu,$$

where P is the average of $D_1^2 - D_1'^2$, $D_2^2 - D_2'^2$, etc., and Q is the average of $D_2^2 - D_1^2$, $D_2'^2 - D_1'^2$, etc.

This method of measurement was found to give very consistent results and is more accurate than the usually adopted method of interpolation with the set of fringes from one side of the plate only.

Experimental Results

In the following, the term nomenclature and the classification of lines are those given by Crawford and McLay (1).

λ5144. Classification $1_0^\circ - 8_1$

The hyperfine structure of this line has already been measured by Fisher and Goudsmit. The $d\nu$'s between the three hyperfine structure levels of 8_1, as measured by the Lummer plates, are given in Table I together with the measures of Fisher and Goudsmit.

TABLE I

OBSERVED STRUCTURE OF λ5144

f	Quartz $\Delta\nu = 0.9115$	Glass $\Delta\nu = 0.8725$		Mean $d\nu$	Fisher and Goudsmit
		Plate 1	Plate 2		
7/2	1.020	1.017	1.020	1.019	1.027 ± 0.007
9/2	0.561	0.563	0.563	0.562	0.564 ± 0.007
11/2	0	0	0	0	0

The ratio of the intervals 0.562 and 0.457 is 1.230, which differs by less than 1% from the value 1.222 predicted by the interval rule.

λ5719. Classification $2_1^* - 7_0$

Table II gives the hyperfine structure intervals of the term 2_1^*.

TABLE II

OBSERVED STRUCTURE OF λ5719

f	Quartz $\Delta\nu = 0.9195$			Glass $\Delta\nu = 0.8815$	Mean of measure with quartz plate	Fisher and Goudsmit
	Plate 1	Plate 2	Plate 3			
7/2	3.908	3.904	3.906	—	3.906	3.895 ± 0.006
9/2	2.143	2.145	2.144	*2.147	2.144	2.140 ± 0.006
11/2	0	0	0	0	0	0

**On the glass plate two components are blended.*

The ratio of the intervals 2.144 and 1.762 is 1.216. This is 0.5% lower than the predicted value 1.222.

λ6600. Classification $1_0^0 - 6_1$

The hyperfine structure of 6_1 has been obtained by Fisher and Goudsmit from the lines λ4705 and 4477. These lines are rather complex, consisting theoretically of nine and seven components respectively, of which eight and six were observed by Fisher and Goudsmit. The line λ6600 involves only hyperfine structure intervals of 6_1, and should therefore give a more direct and accurate measurement of the intervals. These are presented in Table III.

TABLE III

OBSERVED STRUCTURE OF λ6600

f	Glass $\Delta\nu = 0.8909$	Quartz $\Delta\nu = 0.9277$			Mean of measures with quartz plate	Fisher and Goudsmit	
		Plate 1	Plate 2	Plate 3		From λ4705	From λ4477
11/2		2.696	2.696	2.698	2.697	2.71 ± 0.01	2.68 ± 0.04
9/2	*1.197	1.213	1.213	1.215	1.214	—	—
7/2	0	0	0	0	0	0	0

**Two of the components are blended.*

The ratio of the intervals 1.483 and 1.214 is 1.222, in excellent agreement with the interval rule.

λ6808. Classification $2_1^0 - 6_1$

The structure of this line can be predicted with some certainty from the previously measured separations of the 2_1^* and 6_1 terms.

In Table IV, the first column indicates how the components originate; *e.g.*, *e* arises from a transition from the $f = 9/2$ level of 6_1 to the $f = 11/2$ level of 2_1^0. The second column gives the theoretical intensities calculated by the formulas of Hill (4).

TABLE IV

PREDICTED AND OBSERVED STRUCTURE OF λ6808

Change in f	Theoretical intensity	Designation of component	Predicted $d\nu$	Observed $d\nu$			
				Quartz $\Delta\nu = 0.9292$			Glass $\Delta\nu = 0.8927$
				Plate 1	Plate 2	Plate 3	
9/2→11/2	39	*e*	−1.483	−1.476	−1.482	−1.477	
7/2→ 9/2	39	*b*	−0.553	−0.547	−0.552	−0.548	
11/2→11/2	57	*h*	0	0	0	0	0
9/2→ 9/2	1.6	*d*	0.661	–	–	–	
7/2→ 7/2	25	*a*	1.209	1.223	1.220	1.219	
11/2→ 9/2	39	*g*	2.145	2.153	2.149	2.151	
9/2→ 7/2	39	*c*	2.423	2.424	2.421	2.424	2.427

For the quartz plate, *a* and *g* are blended but not quite coincident, but *e* and *b* are practically coincident. In the case of the glass plate, only *h* and *c* can be measured; the other lines form two unresolved groups.

λ5270. Classification $2_1^0 - 8_1$

Using the intervals of the terms 2_1^0 and 8_1 given above, the expected structure of λ5270 was calculated.

The predicted separations between the seven components are given in the third column of Table V, together with the observed values.

TABLE V

PREDICTED AND OBSERVED STRUCTURE OF λ5270

Change in f	Theoretical intensity	Predicted $d\nu$	Observed $d\nu$			Fisher and Goudsmit
			Glass $\Delta\nu = 0.8747$		Quartz $\Delta\nu = 0.9135$	
			Plate 1	Plate 2		
11/2→11/2	57	0	0	0	0	0
9/2→11/2	39	0.562	0.553	0.552	0.559	0.553 ± 0.006
11/2→ 9/2	39	2.144	2.152	2.153	*2.164	2.149 ± 0.004
9/2→ 9/2	1.6	2.706	—	—	—	
7/2→ 9/2	39	3.163	3.177	3.176	3.170	3.166 ± 0.004
9/2→ 7/2	39	4.468	4.474	4.468	4.471	3.995 ± 0.004
7/2→ 7/2	25	4.925	4.927	4.926	*4.906	4.926 ± 0.004

**These components overlap for the quartz plate.*

λ5209. Classification $2_1^0 - 9_2$

Of the nine components of this hyperfine structure multiplet, eight have been observed by Fisher and Goudsmit. The remaining component has been found in the Lummer plate patterns. Fisher and Goudsmit give

$0.125 \pm 1\%$ as the separation factor of 9_2. It is found, however, that the separations in the hyperfine structure multiplet predicted on the assumption that the factor for 9_2 is 0.124 and that the interval rule is valid for the 9_2 hyperfine structure levels, do not agree very closely with the observations. Another value for the factor, 0.121, was assumed. This improves the agreement between observation and prediction for some of the components, but accentuates the differences between the expected and observed separations for other components. It is therefore somewhat doubtful whether the interval rule can be regarded as applying to the term 9_2.

TABLE VI

PREDICTED AND OBSERVED STRUCTURE OF λ5209

Change in f	Theoretical intensity	Predicted $d\nu$. Separation factor of 9_2		Fisher and Goudsmit	Observed $d\nu$		
					Quartz $\Delta\nu = 0.9126$		Glass $\Delta\nu = 0.8736$
		0.124	0.121		Plate 1	Plate 2	
9/2→11/2	26	−1.491	−1.455	−1.477			
*11/2→11/2	*118	−0.809	−0.790	−0.783			
13/2→11/2	335	0	0	0	0	0	0
7/2→9/2	75	+0.095	+0.145	+0.181	—	—	0.193
9/2→9/2	155	0.654	0.690	0.688	0.662	0.666	0.673
11/2→9/2	170	1.336	1.355	1.383	1.353	1.355	1.356
5/2→7/2	144	1.430	1.482		1.428	1.424	1.428
*7/2→7/2	*117	1.858	1.908	1.884			
9/2→7/2	59	2.417	2.452	2.432	—	—	2.420

**These components are blended in the Lummer plate patterns.*

λ4259. Classification $8^\circ_4 - 14_4$

This line would be expected to have 21 hyperfine structure components. Both the quartz and glass plate patterns show six components, of which one is very broad. The measured separations are as given in Table VII.

TABLE VII

OBSERVED STRUCTURE OF λ4259

Component	Observed intensity	Quartz $\Delta\nu = 0.8920$		Glass $\Delta\nu = 0.8559$	Assigned transition	Theoretical intensity
		Plate 1	Plate 2			
ε	2	−0.301	−0.308	−0.304		
κ	1	−0.139	−0.135	−0.136		
α	10	0	0	0	17/2→15/2	236
β	8	+0.133	+0.128	+0.128	15/2→13/2	152
γ	6	0.224	0.227	0.223	13/2→11/2	111
δ (broad)	3	0.343	0.355	0.346		

By using the graphical method described by Fisher and Goudsmit (3) and the intensity relations calculated by the formulas of Hill (4, p. 782) it is found that the data in Table VII lead to a value of B/A equal to 0.7. The assigned hyperfine structure quantum number transitions and the relative intensities are given in Table VII. The values of A and B can now be estimated and they are found to be 0.080 and 0.056 respectively.

λ4272. *Classification* $7_1^\circ - 13_1$.

Fisher and Goudsmit have calculated the separation factors of 7_1° and 13_1 from their observations of the hyperfine structure of combinations of 7_1° and 13_1 with other terms. The factors are 0.099 and 0.065 for 7_1° and 13_1 respectively. Using these values the expected separations of the 15 components are as shown in Table VIII.

TABLE VIII

PREDICTED STRUCTURE OF λ4272

Transition	$d\nu$	Theoretical intensity	Designation	Transition	$d\nu$	Theoretical intensity	Designation
11/2→13/2	−0.906	22	*u*	3/2→ 5/2	+0.035	240	*a*
9/2→11/2	−0.620	61	*q*	7/2→ 7/2	0.079	292	*e*
13/2→13/2	−0.483	194	*v*	13/2→11/2	0.161	646	*s*
7/2→ 9/2	−0.367	112	*h*	5/2→ 5/2	0.197	189	*b*
11/2→11/2	−0.262	300	*r*	11/2→ 9/2	0.283	397	*p*
5/2→ 7/2	−0.149	172	*d*	9/2→ 7/2	0.371	208	*g*
9/2→ 9/2	−0.075	330	*k*	7/2→ 5/2	0.425	75	*c*
15/2→13/2	0	960	*w*				

TABLE IX

OBSERVED STRUCTURE OF λ4272

Observed intensity	Quartz $\Delta\nu = 0.8924$	Glass $\Delta\nu = 0.8562$	Identification of components
5	−0.447	−0.442	*v* and *g* blended
5	−0.206	−0.219	*r* and *d* blended
10	0	0	*w*
7	+0.151	+0.149	*s*
4 sharp	0.297	0.288	*p*

The Lummer plate patterns each gave five components, the separations of which are given in Table IX.

λ4392. *Unclassified*

This line, which as yet has not been classified, was found by Fisher and Goudsmit (3) to have three components. The interference patterns obtained with both the quartz and glass plates also showed this line to be a triplet.

In Table X are given the separations of the components.

TABLE X

OBSERVED STRUCTURE OF λ4392

Quartz $\Delta\nu = 0.8957$		Glass $\Delta\nu = 0.8594$		Fisher and Goudsmit
Plate 1	Plate 2	Plate 1	Plate 2	
0.598	0.602	0.601	0.607	0.64 ± 0.02
0.244	0.251	0.242	0.247	0.30 ± 0.01
0	0	0	0	0

λ4561

The hyperfine structure of this line of Bi III, classified as $7s\ ^2S_{1/2} - 7p\ ^2P^\circ_{1/2}$ has already been measured by Fisher and Goudsmit. The Lummer plate measurements are given in Table XI.

TABLE XI

OBSERVED STRUCTURE OF λ4561

Glass $\Delta\nu = 0.8636$		Quartz $\Delta\nu = 0.8999$	Mean $d\nu$	Fisher and Goudsmit
Plate 1	Plate 2			
2.891	2.891	2.883	2.888	2.88 ± 0.01
2.354	2.351	—	2.353	2.36 ± 0.01
0.534	0.533	0.538	0.535	0.52 ± 0.01
0	0	0	0	0

The hyperfine structure of the $7s\ ^2S_{1/2}$ and $7p\ ^2P^{\circ}_{1/2}$ terms are thus found to be 0.535 and 2.353 respectively, giving separation factors 0.107 and 0.471.

Discussion

The determination of the separation factor of the 14_4 term completes the separation data of the four terms 10_2, 12_3, 13_3 and 14_4 of the $6p_{1/2}5f$ configuration.

In Table XII the observed separation factors A are given, together with the values in terms of the factors for the individual electrons expected if the coupling were (j, j), p'' referring to the $p_{1/2}$ electron, f' and f'' referring to the $f_{3 1/2}$ and $f_{2 1/2}$ electrons respectively.

TABLE XII

SEPARATION FACTORS OF $6p_{1/2}\ 5f$ TERMS

Term		A observed	A for (j, j) coupling
$6p_{1/2}5f_{3 1/2}$	10_2	-0.008	$-a''/6 + 7f''/6$
$6p_{1/2}5f_{2 1/2}$	12_3	-0.018	$a''/6 + 5f''/6$
$6p_{1/2}5f_{3 1/2}$	13_3	$+0.065$	$-a''/8 + 9f'/8$
$6p_{1/2}5f_{3 1/2}$	14_4	$+0.056$	$a''/8 + 7f'/8$

The smallest value given by Fisher and Goudsmit for the 12_3 has been taken. An incomplete analysis of λ4340 from the Lummer plate data indicates that A for 12_3 should be approximately -0.016.

As Crawford and McLay (1) have pointed out, the data for the first three terms indicate that the coupling cannot be regarded as being strictly (j, j), so that it is only appropriate to apply the summation relations. However, the separation factor of the 14_4 term is independent of coupling, so if the well established value $a'' = 0.465$ is taken, f' is found to be -0.002.

The summation relations lead to the following equations

$$A(14_4) + A(13_3) + A(12_3) + A(10_2) = 2(f' + f'')$$

$$A(14_4) + A(13_3) + A(12_3) = \frac{a''}{6} + 2f' + \frac{5}{6}f''$$

$$A(14_4) = \frac{a''}{8} + \frac{7}{8}f' .$$

These equations, together with the data of Table XII, give two values for $f' + f''$, *viz.*, 0.048 and 0.034. These are in as close agreement as could be expected from the limits of accuracy of the data. It might be concluded that the term classification is correct and that the terms are not appreciably perturbed. If the average value for $f' + f''$ is taken, then $f'' = 0.043$.

With the evaluation of the separation factor of the term 8°_3 the data for the four terms arising from the configuration $6p_{1/2}\ 6d$ are completed as set forth in Table XIII.

TABLE XIII

SEPARATION FACTORS OF $6p_{1/2}\ 6d$ TERMS

Term		A observed	A for coupling
$6p_{1/2}6d_{1 1/2}$	5°_1	$+0.127$	$a''/4 + 3d''/4$
$6p_{1/2}6d_{1 1/2}$	6°_2	-0.165	$-a''/4 + 5d''/4$
$6p_{1/2}6d_{2 1/2}$	7°_2	$+0.099$	$-a''/6 + 7d'/6$
$6p_{1/2}6d_{2 1/2}$	8°_3	$+0.080$	$a''/6 + 5d'/6$

It is clear from the data that the coupling cannot be (j,j) but the separation factor of the term 8°_{3}, being independent of coupling, leads to a value of 0.003 for d' if a'' is taken to be 0.465.

The summation relations lead to the following equations:

$$A(8^{\circ}_{3}) + A(7^{\circ}_{2}) + A(5^{\circ}_{2}) + A(6^{\circ}_{1}) = 2(d'_{2} + d'')$$

$$A(8^{\circ}_{3}) + A(7^{\circ}_{2}) + A(5^{\circ}_{2}) = \frac{a''}{4} + 2d' + \frac{3}{4}d''$$

$$A(8^{\circ}_{3}) = \frac{a''}{6} + \frac{5}{6}d'.$$

These equations give two widely different values for the sum $d' + d''$, *viz.*, 0.070 and 0.248. This discrepancy must be attributed to perturbation of the terms 5°_{2} and 7°_{2} by the term 4°_{2}, as suggested by Crawford and McLay (1).

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THE MEASUREMENT OF SOME THERMAL PROPERTIES OF DEUTERIUM OXIDE, AND THEIR INTERPRETATION[1]

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Abstract

Values for the heat capacities of solid deuterium oxide and the resulting liquid, from initial temperatures between 4° C. and −78.5° C. to a final temperature of 25.0° C., have been determined. The specific heats of the solid over the temperature range 0 to −70° C. have been measured. The latent heat of fusion of deuterium oxide has been determined. The specific heat of liquid deuterium oxide is shown to be greater than that of water in the temperature region in which measurements were made. A comparison of the thermal properties of deuterium oxide with those of hydrogen oxide has been made, and certain points of interest are indicated.

Introduction

Some years ago a technique (1, 2) was developed in this laboratory for the measurement of specific heats and latent heats of fusion at moderately low temperatures. An investigation is at present under way to improve the calorimeter so as to ensure a much higher degree of accuracy and, at the same time, extend the temperature range over which precision measurements can be made. As a preliminary, the method previously employed (1, 2) was used for a redetermination of the specific heat of ice. The data previously published were confirmed to within the accuracy stated. As this method is admirably adapted for the purpose, it was considered of interest to measure the thermal properties of deuterium oxide.

Experimental

The procedure described previously was followed closely except that the deuterium oxide was distilled into the platinum container through a platinum tube gold-soldered to the filling tube. A capillary glass tube led into the platinum tube near its end and was cemented into place. The distillation was carried out *in vacuo*, and rigid precautions were taken to avoid contamination of the deuterium oxide by moisture from the air. The deuterium oxide, a 12 gm. sample, was obtained from the Ohio Chemical Company and was 98% pure. The thermostatic control of the required temperatures, the manipulation of the adiabatic radiation thermocouple calorimeter, the corrections and method of calculation have been described (1, 2) and need not be repeated here.

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Results

In Table I are shown the total heat changes of 1 gm. of deuterium oxide when warmed up from various temperatures to 25° C. Each value is the mean of three or more determinations. Thus the determinations at −10° C. gave 103.8, 103.7, 103.9. The accuracy with which the total heats were determined is thus about 0.2%.

TABLE I

HEAT CAPACITIES OF DEUTERIUM OXIDE

Initial temp., ° C.	4	3.8	2	0	−5	−10	−30	−78.5
Heat obs., cal.	21.6		96.26	98.2	101.0	103.8	113.5	134.5
Heat calcd., cal.		95.9		98.0	100.9	103.7	113.9	134.6

The last line contains the values calculated from the empirical equation

$$H = 183.22 - 0.2703\,T + 0.000654\,T^2 - 0.00000296\,T^3 + \frac{2.79 \times 10^{11}}{T^5}.$$

The object of finding such an equation was to facilitate the calculation of the specific heats by differentiation of the equation. The first value in Table I is the heat required to warm up liquid deuterium oxide from 4 to 25° C. The largest number of determinations were made at this temperature, since the heat change is smallest and the accuracy is least. The average specific heat of liquid deuterium oxide is 1.028 over this range of temperature, and this value probably is correct to one-half of one per cent.

The latent heat of fusion can be determined by graphic methods, *i.e.*, by extrapolating the heat curves for solid and liquid and measuring the distance between them at 3.8° C., or by using the above equation for the heat change of the solid and an equation, $H = (25 - t)\,1.028$, for the heat change of the liquid. The first method gives 74.2 and the second 74.0 cal. The determination at + 2° C. was not used in the calculations because at this temperature the solid is so close to its melting point. This has been discussed in an earlier paper (5). The latent heat of fusion, therefore, may be taken as 74.2 ±0.2 cal.

In Table II are given the specific heats of solid deuterium oxide at various temperatures and, for comparison, those of ordinary ice at the same temperatures.

TABLE II

SPECIFIC HEATS OF SOLID DEUTERIUM OXIDE

Temp., ° C.	Specific heat, cal.	
	D_2O	H_2O
0	0.579	0.4873
−10	0.545	0.4770
−20	0.514	0.4647
−30	0.484	0.4504
−40	0.457	0.4340
−50	0.432	0.4160
−60	0.410	0.3958
−70	0.391	0.3737

Discussion

The specific heats of both liquid and solid deuterium oxide are greater than those of ordinary water; the latent heat of fusion however is less. The latter is the only thermal constant of deuterium oxide previously reported.

It was not directly determined but was calculated by La Mer and Baker (4) from the lowering of the freezing point. Their two estimated values, 79.9 and 75.4 cal., are both greater than the value obtained in the present investigation.

As mentioned previously 98% deuterium oxide was used in the present study. Extrapolation to 100% does not change appreciably the values given above. Thus, in the case of the latent heat of fusion it makes a difference of only 0.1 cal.

A comparison of the thermal constants of water and of deuterium oxide on a molecular basis is of interest. The specific heats of solid D_2O are greater than those of ice, so that the molecular heats are still greater. This is to be expected especially if, as present data (3) suggest, they have the same structure with almost the same interatomic distances. In that case the deuterium oxide with its heavier atoms should have the greater molecular heat. When the structure of solid D_2O has been definitely confirmed by X-ray analysis, it may be possible to calculate the relative frequencies of vibration from the specific heat data.

It was pointed out in a recent paper (6) that in liquid D_2O the equilibrium between associated and non-associated molecules $n(D_2O) \rightleftarrows (D_2O)n$ probably is shifted more to the right than in the case of water. With rise in temperature there are more associated $(D_2O)n$ molecules to dissociate, and consequently the molecular heat of the liquid D_2O should be greater than that of water. As shown above even the specific heat is greater.

On the basis of the above equilibrium it might be predicted that conversely the molecular heat of fusion of deuterium oxide should be less than that of ice, because it melts to form a liquid having a higher concentration of associated molecules. On the other hand, the higher temperature at which it melts and the increased mass of the molecules should increase the molecular heat of fusion of D_2O as compared with that of ice. The fact that they are nearly the same, 1484 for D_2O, 1436 for water, shows that the counteracting effects almost balance, and consequently the idea of greater apparent association in liquid D_2O is corroborated.

Acknowledgment

Acknowledgment is hereby made to the anonymous donors of a grant of $1,000 which has made possible this and subsequent calorimetric investigations, one of which will include further work on deuterium oxide.

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HEAT CAPACITY MEASUREMENTS ON GELATIN GELS.[1] III.

BY W. R. HORN[2] AND J. H. MENNIE[3]

Abstract

The heat required to warm a gelatin gel from 0° to 25° C. is greater than the sum of the heat capacities of the water and the gelatin present by an amount which varies with the concentration of the gel, and which equals 6.7 cal. per gram of dry gelatin for gels of concentration below about 52%. It is inferred that there is less bound water, or the water is less firmly bound, at 25° than it is at 0° C. If the heat capacity measurements are plotted against gel concentration, there is a sharp discontinuity at 0.52 gm. water per gram of dry gelatin, which is interpreted as meaning that this is the amount of water which is closely bound at 0° C.

Introduction

In the course of work previously reported (2) it was found that the heat capacity of a gelatin gel between 0° and 25° C. is greater than the sum of the heat capacities of the water and gelatin which it contains. This was attributed to the occurrence, within this temperature range, of some change, accompanied by a heat effect, in the gelatin-water relation in the gel. Furthermore, it was observed that there appeared to be a sudden increase in the heat effect between 61 and 67% gel concentration. It was thought worth while to investigate further by making a number of measurements of heat capacity between 0° and 25° C. on gels of varying concentration.

Experimental

The apparatus and method were the same as previously described (1, 2). The material also was the same, Eastman Kodak Co. ash-free gelatin, Lot No. 48. Gels were prepared as before by dipping the air-dry gelatin in water for a definite length of time, rapidly removing surface water with filter paper and pressing the moist material into the Monel metal container. At the same time duplicate samples were taken for analysis. The gel was then heated for half an hour at 50° C. in the sealed container. A few more dilute gels were prepared by heating the gelatin with the desired amount of water for half an hour at 50° C. and then pouring it into the container. The water content was determined by heating for 24 hr. at 105° C., which was found to be sufficient for the samples to come to constant weight.

The container was kept in an ice-water bath for at least one hour before it was transferred to the calorimeter. The container used was really designed for use with frozen gels where the heat effects to be measured were considerably larger. The temperature drop in the calorimeter was about 0.5° to 0.7°, of which only about one-third was caused by the gel, the remainder being due to the heat capacity of the container. The temperature drop was read on a Beckmann thermometer, and is the determining factor which

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limits the accuracy of the heat measurements. Two or more runs were usually made with each specimen of gel, and the results, which are shown in Table I, represent in most cases the mean of duplicate determinations.

TABLE I

% Gel	Heat capacity per gm. gel, cal.	Gm. water per gm. dry gelatin	Heat capacity of water per gm. dry gelatin, cal.	Gm. gelatin per gm. water	Heat capacity per gm. water, cal.
18.7	22.9	4.35	115.4	0.230	26.5
28.1	22.2	2.56	71.9	0.391	28.1
36.0	20.9	1.78	51.0	0.563	28.7
49.6	19.5	1.02	32.3	0.984	31.8
51.4	19.6	0.945	31.1	1.06	32.9
55.8	18.1	0.792	25.3	1.26	31.9
58.0	17.7	0.724	23.4	1.38	32.3
59.4	17.2	0.683	21.8	1.46	31.9
61.8	16.4	0.618	19.4	1.62	31.4
62.5	16.2	0.600	18.8	1.67	31.3
63.0	15.7	0.589	17.8	1.70	30.3
64.0	15.4	0.563	17.0	1.78	30.2
65.6	16.4	0.524	17.9	1.91	34.1
66.1	16.1	0.513	17.2	1.95	33.5
66.2	15.9	0.510	16.9	1.96	33.1
66.3	15.7	0.508	16.6	1.97	32.7
67.2	14.2	0.488	14.0	2.05	28.7
67.5	14.1	0.482	13.8	2.08	28.7
68.7	14.0	0.455	13.3	2.20	29.2
71.2	13.0	0.404	11.1	2.47	27.4

In Column 2 of Table I is given the total heat capacity per gram of gel between 0° and 25° C., obtained directly from the calorimetric measurements. Multiplying these figures by the weight of gel which contains 1 gm. of dry gelatin and subtracting the heat capacity of the dry gelatin (2) gives the apparent heat capacity of the water associated with 1 gm. of dry gelatin (Column 4). This is represented graphically in Fig. 1. The upper curve covers the range up to 8 gm. of water per gram of gelatin; the lower curve shows on a larger scale the region of the more concentrated gels, up to 2 gm. of water per gram of gelatin. The points previously obtained (2) are shown by the solid black circles. For gels containing more than 1 gm. of water per gram of

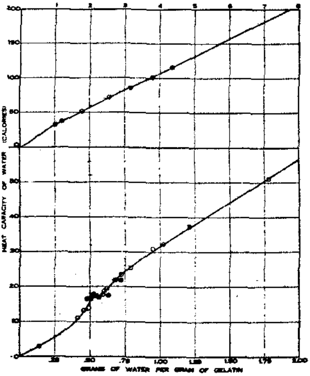

FIG. 1. *Heat capacity of water associated with one gram of dry gelatin in gels of various concentrations.*

gelatin these agree perfectly with the present results. For the three points at 0.72, 0.63 and 0.48 gm. of water, the agreement is less satisfactory although the discontinuity in the neighborhood of 0.5 gm. water per gram of gelatin, which was indicated by these earlier measurements, is now clearly confirmed.

The graph shows the change in heat capacity as increasing amounts of water are added to one gram of dry gelatin. The slope of the curve at any point then indicates the heat capacity of a small increment of the water content of the gel at that concentration. As might be expected, the initial slope is small, corresponding to a specific heat of less than 1 for the first portion of the water absorbed by the gelatin. The slope rapidly increases beyond that corresponding to the heat capacity of ordinary free water. The "apparent heat capacity" of course includes the actual heat capacity of the water and also the heat effect mentioned in the opening paragraph. This heat effect evidently appears in quite concentrated gels, and increases rapidly to a maximum at 0.52 gm. of water per gram of gelatin. It drops then suddenly as far as 0.57 gm. of water, then begins to rise again more gradually. Beyond about 0.9 gm. of water (52% gel) the curve becomes a straight line with slope corresponding exactly to the heat capacity of ordinary free water. At any point on this straight-line portion of the curve, the total heat capacity of the water in the gel is 6.7 cal. greater than that of an equal weight of ordinary free water. That is, the observed heat effect per gram of gelatin has a constant value of 6.7 cal. in gels of less than about 52% concentration.

Evidently, as far as its heat capacity is concerned at least, it is only the water in excess of about 0.9 gm. per gram of dry gelatin which is not influenced by the presence of the gelatin and may be described as "free" water. This is made further apparent by arranging the data in a slightly different manner. Dividing the figures in Column 4 by the weight of water associated with 1 gm. of dry gelatin (Column 3) gives the apparent heat capacity per gram of the water in the gels (Column 6). This is plotted in Fig. 2 against the weight of gelatin per gram of water. As increasing amounts of gelatin are added to a constant quantity of water, the heat effect observed in the gel increases in direct proportion to the amount of gelatin present, up to about 1.1 gm. of gelatin per gram of water. Beyond this point it begins to decrease. Evidently at this concentration (52%) all the water is more or less bound by the gelatin. As the ratio of gelatin to water is further increased, the amount of water held by the gelatin is now less than the maximum and the heat effect, which must arise from some change in the relation between gelatin and "bound" water, also falls off from the maximum value.

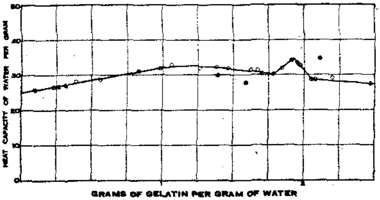

FIG. 2. *Heat capacity per gram of the water in gels of various concentrations.*

Discussion of Results

The precise nature of the changes occurring in the gel is not completely obvious. It may readily be imagined that a rise in temperature might weaken the forces of attachment between water and gelatin; that a portion of the water might be released as the temperature is raised; and that energy would have to be supplied in excess of the amount required merely to effect the increase in temperature. It would follow that in a gel at 25° C. there is less bound water, or the water is less firmly bound than at 0° C. Apparently about 0.9 gm. of water per gram of gelatin is held at 0° C. When the temperature is raised to 25° C. a portion of this is set free with the absorption of 6.7 cal. of heat per gram of dry gelatin. When the gel concentration is greater than this, the amount of water held by the gelatin is less, whence naturally the amount liberated between 0° and 25° C. and the corresponding heat required are also less.

On this basis, to account for the peculiar break in the curve between 0.52 and 0.57 gm. of water per gram of gelatin, it seems necessary to assume the existence of two distinct states or types or degrees of binding, and further, to postulate that the more firmly held portion of the water, which is obviously closest to the gelatin, either cannot escape into the less firmly bound region or, more probably, can do so only to a definite and constant extent, as long as a certain minimum amount of water is present in that outer layer. This minimum is then reached at 0.57 gm. of water per gram of gelatin and the heat effect is also a minimum at this point. When the amount of water is a little less than this, the outer layer of loosely bound water is incomplete at 0° C. and an increased amount of the more firmly bound water escapes as the temperature is raised, with an increase in the observed heat effect. At 0.52 gm. of water per gram of gelatin the outer layer has disappeared entirely and all the water is in the firmly bound state at 0° C. The amount which is released as the temperature is raised is then a maximum. Further increase in gel concentration means a decrease in the amount of water present and a decrease in the heat effect.

The extent of the decrease in the heat effect which occurs between 0.9 and 0.57 gm. of water per gram of gelatin seems surprisingly large if the water in this region is only loosely bound to the gelatin. Moreover Rosenbohm's (4) measurements on the heat of swelling, which were made with a Bunsen ice calorimeter at 0° C., indicated that the liberation of heat is practically complete when about 0.5 gm. of water has been absorbed per gram of gelatin. The results of the present work indicate that the heat of swelling should be less at 25° than at 0° C., the difference amounting to 6.7 cal. when an amount of water greater than 0.9 gm. is added to 1 gm. of dry gelatin. Some measurements of the heat of swelling are now in progress.

Since this work was started, some very interesting results along the same lines have been published (3). The heat of swelling of gelatin has been measured at 18° and at 50° C. A difference in the integral heat of swelling at these temperatures appears for amounts of water more than 0.18 gm. of water

per gram of gelatin. The difference reaches a maximum of about 12 cal. when about 0.6 gm. of water has been added. The authors infer the existence of a heat effect which they call "heat of gelatinization." They attribute it to changes taking place within the gel, of which the sol-gel transformation, which occurs within the temperature range of their investigation, is an outward and visible manifestation. They consider an explanation in terms of alteration in the gelatin-water relation, along the lines suggested in this paper, and dismiss it as being of, at most, secondary significance, on the ground that the heat effect observed is greater than is to be expected on the basis of changes in the "water-mantle" of the gelatin. They regard the principal source of the heat effect as being more probably a transformation from one modification of gelatin to another as the temperature is raised. They quote evidence from optical rotation and X-ray measurements which suggests the existence of such a transformation.

They conclude from their results that the temperature at which this transformation begins depends on the water content of the gel. At 0.230 gm. of water per gram of gelatin it begins only at 30° C.; at 0.6 gm. of water per gram of gelatin it begins at 15° and is complete at 40° C. This appears to be in general agreement with the present work, although their curves show no sign of the discontinuity between 0.52 and 0.57 gm. of water, which is so striking a feature of the results reported here.

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THE CATALYTIC DEHYDRATION OF ETHYL ALCOHOL BY ALUMINA

I. THE EFFECT OF THE WATER CONTENT OF THE CATALYST[1]

By L. A. Munro[2] and W. R. Horn[3]

Abstract

The dehydration of ethyl alcohol has been studied at 250° C. using alumina catalysts differing in water content. There is an optimum water content for the greatest activity of the catalyst. The apparent poisoning is greatest for those catalysts having greatest activity. There is no apparent poisoning for a catalyst having zero water content. In no case was the course of the reaction changed.

Introduction

The decomposition of ethyl alcohol may proceed in three ways—

(1) $C_2H_5OH \longrightarrow CH_3CHO + H_2$
(2) $C_2H_5OH \longrightarrow C_2H_4 + H_2O$
(3) $2C_2H_5OH \longrightarrow (C_2H_5)_2O + H_2O.$

Adkins and Krause (1) consider that the size of the pores in an oxide catalyst will determine to a large extent the type of reaction induced; *i.e.*, whether it will be dehydrogenation, dehydration or decarboxylation in the case of an ester.

Several workers have reported that a change in structure of alumina takes place between 200 and 250° C. Accordingly, in the activation of the gel at temperatures above 250° C. a change in the specificity of the catalyst might be induced.

Engelder (2) has studied the effect of added water on the reaction. He found that the presence of water in the alcohol was unfavorable to dehydration, and when present in large amounts, induced dehydrogenation. The results of Munro and Johnson (7) on the sorption of ether by alumina, and of Munro and McCubbin (8) on the catalytic reaction of carbon disulphide and water, would lead one to expect an *optimum* water content of the alumina for catalysis.

It seemed of interest, therefore, to study the behavior of a typical dehydration catalyst when its water content and the temperature of activation are varied.

Materials

The alumina used was the "moist" type supplied by the British Drug Houses. The lumps were dried for eight hours at 110° C. and then broken up to 10 mesh. Activation was carried out by heating the catalyst tube and contents in an electric furnace for two and one-half hours, at a constant temperature determined by means of a thermocouple. During activation

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and cooling, a stream of nitrogen was passed through the catalyst. The residual water content of the sample was determined by weighing the catalyst before and after activation. The total water content was determined by blasting a sample for 40 hr. over a Meker burner. In Table I are shown the activation temperatures and residual water contents.

TABLE I

ACTIVATION TEMPERATURES AND RESIDUAL WATER CONTENTS

Activation temp., °C.	300	320	410	450	500	550	580
Residual water, %	13.8	12.7	8.5	5.5	4.3	2.3	1.4

NOTE:— *Time of heating, 2½ hr. The residual water content was reduced to 0.0% by blasting with a Meker burner for 40 hr.*

Commercial 95% ethyl alcohol was first refluxed with freshly precipitated mercuric oxide to remove traces of aldehydes. This was followed by two reflux treatments, of several hours each, with quicklime. The product obtained was well over 99% ethyl alcohol.

Experimental Method and Apparatus

The general method consisted in passing a regulated flow of the vaporized alcohol (25 cc. in 90 min.) over the catalyst kept at constant temperature, condensing the liquid reaction products and unchanged alcohol, and collecting any residual gas over saturated brine.

The apparatus is shown in Fig. 1. After trying numerous methods for the introduction of the liquid, it was found that a long burette, *L*, was very satisfactory when the top 25 cc. was used. The preheater, *H*, in the form of a spiral, was placed in a small furnace the temperature of which was maintained 10° C. higher than that of the catalyst. The latter was kept at 250 ± 1° C. by a DeKhotinsky regulator, *R*. The catalyst tube, *C*, was made of 12 mm. Pyrex tubing, 24 cm. long, each end being sealed to 4 mm. tubing. Similar amounts of catalyst were used in each run.

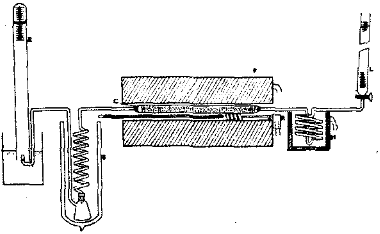

FIG. 1. *Diagram of apparatus.*

Numerous investigators have used a salt freezing mixture for the condensation of the liquid products. With this method the loss of some of the ether from the receiver is probable, especially if gas is evolved. The use of solid carbon dioxide reduced this loss to a negligible amount. The tube *E*, filled with saturated brine, was provided for collecting any gaseous reaction products.

Analysis of the Liquid Reaction Products

Since the ether in the reaction products is mixed with unchanged alcohol, its quantitative estimation presents some difficulty. Extraction with solvents involves losses. The salting-out method used by Pease and Yung (9) is not quantitative when the ether content is less than one-third of the total liquid mixture.

The method of Kunke (6) was used with slight modifications. The ether vapor was entrained in a regulated current of air, the alcohol absorbed in 50% sulphuric acid contained in two gas washing bottles, and the ether oxidized quantitatively by acid dichromate in a Milligan bottle. The excess of dichromate was then titrated iodometrically. By careful manipulation of the process it was possible to check an analysis to within 0.1%.

Results

Curve *A*, Fig. 2, shows the different amounts of ether produced when catalysts of different water content are used. If the alumina contains more than 13.8% of water it has no effect on this reaction. If it contains 13.8% of water a trace of ether can be detected in the reaction products. As the amount of residual water in the catalyst is decreased, the activity increases until at a water content of about 5.5% the conversion to ether reaches a maximum. As the water content of the alumina is decreased further, the activity is lessened and falls steadily, becoming much lower when the catalyst is anhydrous.

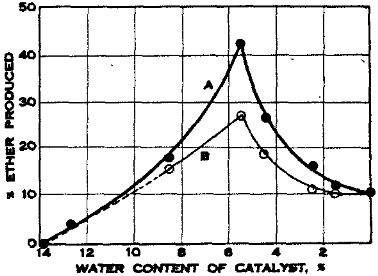

FIG. 2. *Graphical representation of the activity of alumina catalysts containing different amounts of residual water. A, originally; B, after the passage of 50 cc. of ethyl alcohol.*

Other workers have shown that the amount of catalyst used has a noticeable effect on the amount of ether produced, provided that the rate of flow is kept constant. It seems probable that this is simply an observation of the extent to which the catalyst has been poisoned. With a large amount of catalyst, 25 cc. of alcohol may be supplied before the activity is appreciably reduced by poisoning. In this study a comparatively small amount of catalyst (13 gm.) was used, and the decrease in activity is quickly noted. Curve *B*, Fig. 2 represents graphically the activity of the catalysts after the passage of 50 cc. of alcohol.

As will be seen from the curve, the lowering in efficiency is relatively greatest for the catalyst originally having the highest activity. The two curves converge, *i.e.*, the relative poisoning decreases until in the catalysts containing

no residual water, no difference in activity could be detected. The curves also appear to converge at zero activity (13.8% water). In no case was any gas produced.

Discussion

Though adsorptive capacity does not always parallel catalytic activity, nevertheless adsorption does play an important role. In catalysts of high water content, the forces causing adsorption are evidently saturated by the water already present. When activation brings these forces into play again, adsorption and catalysis can begin, although not necessarily at the same point of dehydration (8).

It has been suggested by several investigators that decrease in activity with time is due to auto-toxic adsorption of the reaction products. Thus Ipatiew (5) states that sorbed water slows down the catalytic dehydration of ether, and Engelder (2) has noted a similar effect in the dehydration of alcohol. This would explain why the most active catalysts are poisoned to the greatest extent. It does not, without amplification, explain why no decrease in activity was found with catalysts containing no residual water. Furthermore, Guichard has recently shown (4) that at 250° C., though the adsorption of alcohol is appreciable, *that of ether and water is practically zero.* The maximum in the curves probably does not represent the point of greatest adsorption of alcohol, but rather that point at which the relative number and arrangement of alcohol molecules and alumina groups or atoms are most favorable for the reaction. Beyond this optimum, increased adsorption of alcohol would be unfavorable.

Adkins has contended that structural changes are brought about by heating and may sensibly affect the specificity of a given catalyst. In the present study no measurable amount of ethylene or hydrogen was produced. There was therefore no change induced in the course of the reaction. Either the different activation temperatures and resulting variation in residual water content cause no change in structure, or such a change does not affect the specificity of the catalyst.

An explanation for the foregoing experimental results has been suggested, but it awaits substantiation by further investigation.

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NITROUS OXIDE AS AN OXIDIZING AGENT IN THE GASEOUS STATE[1]

By E. W. R. Steacie[2] and R. D. McDonald[3]

Abstract

The kinetics of a number of gaseous oxidation reactions have been investigated, using nitrous oxide as the oxidizing agent in place of oxygen. It is found that, in general, nitrous oxide is much less efficient as an oxidizing agent than is molecular oxygen. Nitrous oxide in most cases acts merely as a reservoir of atomic oxygen at temperatures where its rate of decomposition is appreciable.

Introduction

In view of the progress that is being made in the study of the kinetics of gaseous oxidations by molecular oxygen, it is of interest to extend the field of investigation to include similar reactions in which oxygen is replaced by other gases.

Dixon and Higgins (6) measured the temperatures of spontaneous ignition of jets of different combustible gases in an atmosphere of nitrous oxide, and found that these temperatures were usually slightly lower than those obtained with oxygen. Since such experiments involve great uncertainty as to gas composition, they cannot yield definite kinetic conclusions, but they seem to indicate that the mechanisms of the two oxidation processes are similar.

In the case of the hydrogen–nitrous oxide reaction, Hinshelwood (9) concluded that the rate of reaction was no greater than could be accounted for by the decomposition of nitrous oxide followed by rapid reaction of the oxygen and hydrogen. Melville (11) has recently carried out an extensive investigation of this reaction. His results showed that the reaction was from 90 to 500 times as fast as the nitrous oxide decomposition, depending on the experimental conditions. His results are satisfactorily explained by a chain mechanism, *the chains being initiated by atomic oxygen formed by the decomposition of* nitrous oxide.

It seems desirable to extend such investigations to other systems, and this paper deals with the oxidation of a number of substances by nitrous oxide.

I. Methyl Alcohol

The methyl alcohol–oxygen reaction has been investigated by Fort and Hinshelwood (7) This is a typical chain reaction, and it therefore seemed worth investigating the nitrous oxide–methyl alcohol reaction.

The apparatus was similar to that used in previous investigations (8, 14). The results obtained have already been described in detail (15). It was found that the reaction proceeded at a conveniently measurable rate at 500 to 570° C.

[1] *Manuscript received January 31, 1935.*
Contribution from the Physical Chemistry Laboratories, McGill University, Montreal, Quebec, Canada.
[2] *Assistant Professor of Chemistry, McGill University.*
[3] *Holder of a fellowship under the National Research Council of Canada at the time when this work was performed.*

This is a much higher temperature than is required for the methyl alcohol–oxygen reaction. At such temperatures the observed rate is much faster than the rate of decomposition of nitrous oxide. It is therefore not possible to ascribe the reaction to a simple decomposition of nitrous oxide followed by oxidation of alcohol by the oxygen thus produced.

The decomposition of nitrous oxide is, however, appreciable in this temperature range. It therefore appears probable that the reaction is a chain process in which the chains are initiated by oxygen atoms derived from the nitrous oxide decomposition. The behavior is therefore similar to that found by Melville for the hydrogen–nitrous oxide reaction.

II. Ethylene

The ethylene–oxygen reaction proceeds at a measurable rate at 300 to 450° C. (*3, 10, 16, 18*). The nitrous oxide–ethylene reaction was found to be slow even at 530° C. At this temperature a large amount of tar and other condensable materials was formed. The main reaction seemed to be polymerization and decomposition of ethylene, followed by a slow oxidation of the products by nitrous oxide. On account of the fact that the non-volatile products formed reacted slowly with nitrous oxide, it would have been necessary to use a new reaction bulb for every run, and the investigation was therefore abandoned.

It was, however, definitely established that nitrous oxide was a very much less efficient oxidizing agent than oxygen, and that it is effective only at temperatures at which its decomposition is becoming appreciable.

III. Acetaldehyde

The acetaldehyde–oxygen reaction proceeds at quite low temperatures, *viz.*, 60 to 120° C. (1, 2, 8, 12).

About 40 runs were made with acetaldehyde–nitrous oxide mixtures. The reaction proceeded at a conveniently measurable rate at 450° C. It was accompanied by a pressure increase of about 115%. The reaction is complicated by the fact that at 450° C. the decomposition of acetaldehyde is fairly large, and depending on its partial pressure from 5 to 20% of the acetaldehyde disappearing does so by decomposition rather than oxidation.

The actual N_2O–CH_3CHO reaction is complex. It is slightly retarded by packing the reaction vessel, the rate diminishing about 20% when a 200 cc. silica bulb is filled with 1 in. lengths of $\frac{1}{8}$ in. tubing. The reaction is therefore a chain process. The products correspond in the main to oxidation to acetic acid followed by the decomposition of the acid to methane and carbon dioxide.

The kinetics of the reaction are complicated. For mixtures having the composition 1 CH_3CHO + 0.7, 1, 2, and 4 N_2O, the time to quarter-value passes through a minimum with increasing pressure for each mixture. For all mixtures T_{25} is between 10 and 15 min. at partial aldehyde pressures of about 5 cm. It decreases to a minimum of about four minutes at pressures between 7 and 10 cm., and then increases again. This results in rather complicated relations for the effects of the concentrations of the separate reactants.

Thus at low aldehyde pressures T_{25} is approximately inversely proportional to (N_2O), at intermediate pressures it is independent of it, and at high pressures it is proportional to it. At low nitrous oxide pressures T_{25} is inversely proportional to (CH_3CHO), while at higher pressures it is independent of (CH_3CHO).

On account of the complicated nature of the reaction, and the fact that the acetaldehyde decomposition causes serious errors in the results, it is not worth while reporting the experimental data in detail. There is, however, no doubt that nitrous oxide does not react with acetaldehyde at all at temperatures where the acetaldehyde–oxygen reaction would proceed practically instantaneously.

IV. Phosphine

The phosphine–oxygen reaction proceeds explosively at room temperature (within certain pressure limits) (4, 5, 19). With phosphine–nitrous oxide mixtures it was found that a 4 N_2O + 1 PH_3 mixture at 600° C. gave a pressure decrease of about 30% relative to the phosphine. Even at this high temperature, however, the reaction was fairly slow, T_{50} being about five minutes for total pressures of 20 cm. At 600° C. and total pressures of about 32 cm. explosions occurred. It is apparent that here again preliminary dissociation of nitrous oxide is necessary.

V. Carbon Disulphide

The carbon disulphide–oxygen reaction also possesses critical pressure limits, and within these explosions occur at temperatures as low as 140° C. (13, 17). The reaction between carbon disulphide and nitrous oxide is negligibly slow at temperatures below 600° C.

Discussion

As mentioned above, Dixon and Higgins found uniformly lower ignition temperatures in nitrous oxide than in oxygen. The variety of reactions which we have investigated show, however, that nitrous oxide is a very inefficient oxidizing agent compared with oxygen. In general, oxidation by means of nitrous oxide occurs only at temperatures at which its dissociation is becoming appreciable. This result, however, is not incompatible with the work of Dixon and Higgins. The substances they investigated all had fairly high ignition temperatures. At such temperatures the decomposition of nitrous oxide is rapid, and the oxidation is really occurring through atomic oxygen.

It may therefore be concluded that, in general, nitrous oxide does not itself act as an oxidizing agent in the gaseous state. It functions merely as a reservoir of atomic oxygen when the temperature is sufficiently high.

Acknowledgment

We wish to express our indebtedness to the National Research Council of Canada for a fellowship awarded to one of us (R. D. McD.) during the tenure of which this work was performed.

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STUDIES OF WOOD

I. THE CELL WALL[1]

BY R. DARNLEY GIBBS[2]

Abstract

A brief review of literature relating to the nature of the cell wall is presented. The difficulty of separating lignin from cellulose is stressed, and attempts to effect separation by the use of mild solvents are described. The torus, pit membrane and border of bordered pits are more resistant to solution than the rest of the wall. The possibility of enzymatic analysis of the wall is noted.

General Introduction

In 1928 co-operation in work on the chemistry of the cell wall was requested by the Department of Cellulose Chemistry at McGill, and later, in 1929, inquiry was extended to the problem of sinkage of logs during the "drive" from woods to mill. The work would have been impossible without the following aids:

a. Financial assistance from the Canadian Pulp and Paper Association through the Department of Cellulose Chemistry at McGill University, and from the Woodlands Section of the Association through its Forester, Mr. A. Koroleff.

b. Grants to cover research and traveling expenditure from the National Research Council of Canada.

c. The co-operation of Price Bros. and Co. Ltd. and of the Canada Paper Company in providing material, hospitality and assistance in the field.

The writer desires to express his gratitude to all of these. He extends it also to Professor Lloyd, in whose Department much of the work has been done, and to Professor Scarth who introduced the writer to the work and followed it with interest and very material aid; to Messrs. Jago and Walton of Price Bros.; to Dr. Hibbert of the Department of Cellulose Chemistry, and to Mr. Cleveland Morgan, who allowed use of his trees at Ste. Anne de Bellevue for experimental purposes.

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Contribution from the Department of Botany, McGill University, Montreal, Canada, with financial assistance from the National Research Council of Canada. From a thesis approved for the degree of Doctor of Philosophy in the University of London, England.

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This work falls naturally into three loosely related sections, of which the present paper constitutes the first. Later papers will deal with studies on the water content of certain (living) Canadian trees, on the changes in water content of logs during seasoning and flotation, and on the physiology of the tree with special reference to the ascent of sap and the movement of water before and after death.

The earliest cell walls, "middle lamellae", probably consist, as Mangin (38) suggested, very largely of pectic compounds. It is true that Tupper-Carey and Priestley (65) considered protein to be present in young walls of *Vicia faba* and *Phaseolus*, but Wood (67), using the "chloramine" reaction, claims that there are only extremely small amounts (0.001% or less) of protein in cellulose walls.

Recent work has widened our knowledge of the pectic compounds to a point where we may be fairly confident as to their chemistry though still somewhat confused as to their position and role in the cell wall. Among those who have contributed to this work we may mention Candlin and Schryver, Carre and Haynes, Conrad, Fellenberg, Nanji and Norman, Nanji, Paton and Ling, Norris, Norris and Schryver, and O'Dwyer. Much of their work is reviewed by Onslow (43). Apparently the materials in question are fairly easy of removal from the *young* cell wall by a variety of solvents and this results in a separation of the cells.

All the usual solvents for pectic compounds—boiling water, 0.5% oxalic acid, 0.5% ammonium oxalate before and after treatment with dilute hydrochloric acid—had very little effect upon microtome sections of spruce, pine, balsam, birch, lime, etc., which retained their strength and appearance even after prolonged treatment, so the middle lamella here can hardly be of pectic nature.

O'Dwyer (41, 42), however, has found small amounts of pectic materials in the woods of beech and oak, so it is evident that they are not entirely absent from lignified tissue. The idea of Candlin and Schryver (10), that a decarboxylation resulting in the formation of hemicelluloses may occur during lignification, might explain the apparent disappearance of pectins (see also (43), p. 86), but a change to hemicelluloses is quite insufficient to explain the behavior of the lignified wall, though the idea is of some interest in connection with the possibility that hemicelluloses are reserve foodstuffs. Chemical relation between pectin and lignin through hemicelluloses has been advanced as a possibility by O'Dwyer (42).

It has been shown by Ritter (51) that the mature woody wall behaves as if the middle lamella is composed of lignin rather than pectin. In his 1925 paper Ritter presented figures indicating that 75% of the lignin of wood is in the middle lamella, but Scarth, Gibbs and Spier (56) pointed out that this figure is impossibly high, the volume of middle lamella being far too small to account for so much lignin. Ritter himself appeared to think his results high and in a later paper (53) gave a lower figure. Harlow (19), in a brief review of the literature, quotes Haberlandt and Rhoads as recognizing that the middle lamella may be lignified, while Schellenberg (58) appears to have

noted this as long ago as 1896. Harlow sees two possibilities:—change of pectins into lignin, or the complete incrustation of pectins by lignin to render them insoluble. He ignores the possibility of the actual removal of pectins during lignification, or their transformation into hemicelluloses, as mentioned above. In a later paper (21) he described a method (chlorine water followed by hot 3% sodium sulphite) for the maceration of woody tissue, which is made possible by the lignin-like character of the middle lamella.

Let us turn now to a brief consideration of cellulose and its role in the cell wall.

A review by Schorger (59) of the theories as to chemical structure of cellulose appeared some eight years ago. In it Schorger compares the formulas of Haworth and Leitch (1919), Hibbert (1921), Irvine (1922-3), Karrer (1921) Karrer and Smirnov (1922), Hess (1923), etc. Most of these workers endeavored to explain the properties of cellulose by the postulation of ring structures composed of from two to four glucose or near-glucose units, but use of the polarizing microscope and the X-ray has resulted in the straight-chain* hypothesis. The papers of Herzog and Jancke (28), Sponsler (61-64), Clark (12), Preston (47), Zeidenfeld (68), Meyer (39) and Astbury, Marwick and Bernal (2) deal with various aspects of the subject.

The present position may be stated as follows: The almost theoretical yields of glucose in total, and the high yields of cellobiose in partial hydrolysis of cellulose lead to the suggestion that glucose in the form of cellobiose (β-glucosido-4-glucose) units forms the basal unit of the wall, while the results of optical (especially X-ray) analyses would appear to confirm this theory. The last has made it possible to calculate the space lattice in the wall, and the unit structure (longitudinal axis 5.15Å, radial spacing 5.33Å, tangential spacing 6.10Å (63)) that is indicated agrees very well with the conception of cellobiose aggregates. One of the greatest difficulties encountered in the X-ray work has been the selection of suitable material. Both Sponsler and Preston have found the wall of *Valonia* to be almost perfect material. Sponsler concluded that while the plane of "spacing" 5.33Å is perpendicular, or nearly so, and the plane 6.10Å roughly parallel to the wall, the cellulose-chain axis (5.15Å) is diffuse, the chains being arranged at various angles. Preston agreed with this finding, but Astbury, Marwick and Bernal (2), using single layers of wall instead of blocks of many layers as employed by Sponsler, have shown that there are ". . . two main sets of cellulose chains which form crystallites crossing at an angle which is maintained remarkably constant through the whole thickness and over considerable areas of wall." Further, these chains are parallel to the microscopically visible striae and their positions and relative numbers account for the behavior of the wall under polarized light.

Not only can the presence and orientation of chains be demonstrated but good evidence for the actual length of the chains may be obtained (1). It seems that *endless* chains would give 2, 3, 5-trimethyl glucose alone under carefully controlled methylation, while the *ends* of chains would give tetra-

**Each glucose residue is cyclic.*

methyl glucose. Actually 0.6% of tetra-methyl glucose has been obtained and this would correspond to a length of 200-300 glucose units (100-150$\mu\mu$). The molecular weight of something over 30,000 calculated for this agrees well with X-ray, Svedburg ultra-centrifuge and Staudinger viscosity measurements, so we find all the evidence pointing in the same direction, even for such widely different structures as cotton fibres and tunicate mantles (39).

It is convenient at this point to consider the "micellar" character of the wall. Cellobiose chains of 200-300 glucose units would have a length of about 100-150$\mu\mu$ and a thickness of about 0.5$\mu\mu$, though the figures given by Seifriz (60) suggest a length of 60$\mu\mu$ corresponding with a chain of fewer units. Meyer supposes that bundles of about sixty of the larger chains are combined with each other to a crystallite having lattice structure (the micellae) such as those of Seifriz (Fig. 1, C). The last considered that "The orientation of the crystallites is confined to the fact that the long axes are approximately parallel; aside from that, they may have any position or orientation and decidedly lack the regularity of bricks in a wall".

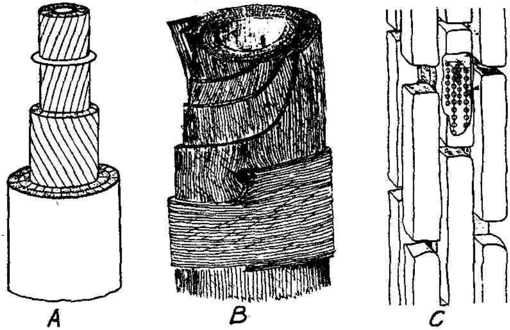

FIG. 1. *Structure of the cell wall. A. Cotton hair (after Meyer). B. Wood fibre (after Scarth in Scarth, Gibbs and Spier). C. Cellulose micellae (after Seifriz).*

The micellae referred to above, with a length of 100-150$\mu\mu$ and width and thickness of the order of perhaps 5$\mu\mu$, would not be visible even when swollen, but there is abundant evidence from microscopic work that the cell wall possesses a micellar or fibrillar structure which is visible under certain conditions (see Ritter (53), and Harlow (19). This would seem to imply the aggregation of these smaller bundles into larger ones, as suggested by Herzog and Jancke (28). The long axis in all but the outer layers would run almost parallel to the fibre length, while in the outer layers the direction would be almost at right angles to this (see Fig. 1 and compare the behavior of fibres on swelling as described by Ludtke (36)).

In addition to longitudinal orientation there is a more or less transverse cleavage structure that is manifested in the "checks" often visible in fibre walls. These form a more or less definite angle (slightly less than 90°) with the longitudinal striae, and observation of their behavior during solution indicates that they run in definite series. These checks, taken in conjunction with the longitudinal cleavage planes, divide the wall into bricks that are probably much more regularly arranged than Seifriz appears to think. The formation of visible micellae, as we have seen, would render some such structure necessary.

The α, β and γ-forms of cellulose are considered to differ only in degree of dehydration or condensation, while oxy-cellulose differs from the normal in that it reduces Fehling's solution, has acid properties, and yields furfuraldehyde on distillation. This is understandable if the free $-CH_2OH$ group of cellobiose be oxidized to $-CHO$ and $-COOH$ groups (in different molecules, of course) and carbon dioxide split out of some of the latter to give xylose units (see de Chalmot (11) and Parsons (44)). Reports that celluloses from cotton and wood are different may perhaps be explained on similar grounds.

Lignin has always presented difficulties and it is idle to pretend that much is known even now as to its origin, structure and role in the cell wall. It cannot be referred, like cellulose, to relatively simple substances, nor can it be isolated with the complete confidence that it remains unchanged. Harlow (19, 21) points out that "lignin" of the botanist may not be the same thing as lignin of the chemist. It is customary for the former to regard walls as "lignified" when they give certain color reactions with such reagents as aniline sulphate, phloroglucin-hydrochloric acid, etc., while the latter classes as lignin material that is removable by chlorine-sulphite treatment or material left after removal of cellulose, etc. by acid. It has long been suspected that the color reactions are due to small amounts of substances of an aldehyde nature occurring *with* lignin but not necessarily a part of it (see Onslow (43), pp. 109-112). Klason (32) gives a formula for α-lignin of spruce which suggests that it is derived from two molecules of coniferyl aldehyde (which gives the phloroglucin reaction), but it cannot be claimed that this formula is definitely established. Harlow (22) has given much attention to the question of staining reactions of lignin and concludes that they are not altogether reliable, while Scarth, Gibbs and Spier (56) also investigated the correlation between staining and (chemists') lignin and found it to be close but not absolute. An extensive review of lignin chemistry is given in a recent paper by Phillips (45).

Attempts to correlate microscopical observation and chemical treatments of wood are of interest. Some of Ritter's observations have been quoted above. In other papers (50, 52, 53) he describes work which, in his opinion, indicates differences between hard- and soft-wood lignin, between amounts of lignin in heartwood and sapwood, and spring and summer wood (Ritter and Fleck (55)), and so forth. He describes "fusiform bodies" from lignin that may be identical with similar structures described by Kürschner (34) as formed

by fungus action on wood. Some of his results have been questioned by Harlow (19, 20, 21, 23, 24) who considers the "cell-wall lignin" of Ritter to *consist of ". . . the charred decomposition products of the polysaccharides of* the cell wall in addition to fragments removed from the lignin blocks by prolonged boiling". Harlow (23) notes, as did Scarth, Gibbs and Spier (56), and Ludtke (37), the different behaviors of the secondary walls of hardwoods and softwoods. He finds (24), however, that there *is* lignin in the secondary walls of hardwoods and that it may retain the form of the original cell after removal of cellulose. Although his chemical tests—complete solution as a result of several treatments with bromine followed by weak ammonia—would seem to indicate removal of all cellulose, it is difficult on viewing his photographs to convince oneself that such is really the case. Freudenberg, Zocher and Dürr (16), and Fischer and Lieske (15), however, also claim that lignin retains the shape of the cell wall after removal of carbohydrates, though considerable shrinkage may occur.

The usual solvents for lignin and cellulose are too drastic in their action, and workers are tending more and more to the use of milder solvents. Work is hampered by the lack of knowledge of the chemistry of lignin, and it is not even known for certain whether lignin and cellulose are in chemical combination. In this connection we may note that lignified walls are said to give the same X-ray picture as pure cellulose walls (17, 18). This would imply that lignin is between the micellae rather than part of them. The fact that color reactions for cellulose may not be given until after fracture of the lignified wall may also be in harmony with this view.

The use of milder solvents is to be commended on chemical grounds and is absolutely essential for microscopic work. In an effort to avoid the swelling and charring resulting from the use of 72% sulphuric acid, graded concentrations (on the principle used in cytology) were employed. This method had its advantages, and it proved possible to dissolve away all but the structurally unaltered (?) middle lamella from 15μ sections. (Some lignin was, of course washed away rather than dissolved.) Even with very slow solution, however, there was considerable darkening and attention has therefore been given to milder solvents* in an effort to isolate the lignin or cellulose unchanged. Among the solvents used were:—

(a) *For cellulose:* Sulphuric acid (all concentrations up to 72%); choral-pyridine; trichloracetic acid (at various concentrations in ether, water and dioxane solutions).

(b) *For lignin:* Lactic acid; glycerol; glycol; aqueous glucose; chlorine in carbon tetrachloride, followed by ammonia; triethanol amine; methyl alcoholic sodium hydroxide (2%).
The first four were used alone, with 0.5% hydrochloric acid, with a trace of iodine, or with 0.5% hydrochloric acid and a trace of iodine. The

**Dr. H. Hibbert very kindly suggested and supplied some of the solvents tried. Mr. C. W. Argue also made a number of tests,—results unpublished.*

last was employed in the cold, following the technique used by Beckmann, Liesche and Lehmann (5) on rye straw. The alcohol is said to prevent solution of hemicelluloses.

(c) *For pectic constituents:* $N/30$ HCl, followed by ammonium citrate as used by Conrad (13); ammonium oxalate; boiling water for long periods.

The technique employed varied greatly with the reagents. Some were used at room temperature only, others at controlled temperatures ranging from about 20–110° C. In a few cases, as when trichloracetic acid and triethanolamine in ethereal solution were tested, the sections were sealed up in small vials and heated at 50–60° C. for various lengths of time.

The sections were obtained from *Alnus rubra*, *Tilia americana*, *Picea* spp., *Betula alba*, *Ochroma lagopus* (Balsa), and *Populus tremuloides*. Most of the solvents listed above were tried on all species, but the use of triethanol-amine and trichloracetic acid was confined for the most part to *Picea alba* and *Populus tremuloides*.

In almost all cases the sections proved very resistant to the solvents and retained their form and strength but little altered. Exceptions were: Sulphuric acid (not a mild solvent) and trichloracetic acid for cellulose, and lactic acid for lignin. Trichloracetic acid appeared to be fairly effective as a cellulose solvent but it was very difficult to be sure that all cellulose had been removed, even after treatments extending over several weeks. As representative of the type of result obtained, two experiments may be quoted.

1. Extraction of Lignin by Glycol

Sections were extracted with hot alcohol-benzene mixture, transferred to pure glycol, to glycol plus a trace of hydrochloric acid, or to glycol plus sufficient iodine to give a pale, straw-colored solution, and extracted at 110° C. for six to eight hours (Hibbert and Rowley (31), Hibbert and Marion (29), Hibbert and Phillips (30)). The solutions were then replaced, by slow stages, by water and the sections were mounted in phloroglucin-hydrochloric acid or in aniline sulphate-sulphuric acid. All gave "lignin" color reactions (red and yellow respectively) but somewhat less strongly than unextracted material, and all had retained their form and were still strong enough to handle. Gradual transfer to 72% sulphuric acid, to remove cellulose, did not result in complete solution, a network of material remaining which consisted largely of middle lamella. This residue (presumably lignin unextracted by the glycol) was subjected to treatment in cold 2% methyl alcoholic sodium hydroxide. After 48 hr. the network appeared to be but little changed.

According to Hibbert and his co-workers, glycol may extract up to 40% of the total cell wall material, but the actual "glycol lignin" recoverable is but a fraction of the total lignin. It is clear from our experiments that the middle lamella at least is but little affected either by the glycol or by the alcoholic sodium hydroxide.

2. Extraction of Lignin by Lactic Acid

According to Hibbert and Phillips, lactic acid containing 0.5 gm. hydrochloric acid per 100 gm. of wood meal extracts as much as 42% of the total material, while the lignin recoverable may amount to 14.1%*. This is a very large proportion of the total lignin and removal of this amount should have a profound effect on the microscopic appearance of wood.

In order to test this, spruce sections were extracted for about six hours at 110° C. with lactic acid-hydrochloric acid and marked maceration of the tissue was observed. The residue no longer gave the aniline sulphate or phloroglucin reactions, and on treatment with 72% sulphuric acid, almost complete solution occurred. There remained, however, *a thin lamella from the cells of the medullary rays and the pit-membranes, tori, and in some cases the arched "borders" of the pits* (Fig. 2).

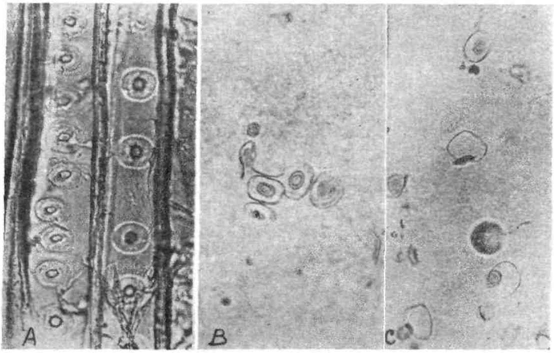

FIG. 2. *A. Radial longitudinal section of spruce to show bordered pits. B. Isolated bordered pits (see text). Note torus at top left. Same magnification as A. C. The same. In three cases the tori are free from the borders which appear as colorless rings. Same magnification as A and B.*

The isolated tori with the pit membranes reminded one strongly of the figures given by Bailey (3) and, in spite of the difficulty of observation, one could just distinguish in a few cases the pores (?) of the membranes as elongated radiating areas. Sometimes the membrane appeared to be dissolving from the edge and a rather regular raggedness, the indentations of which might correspond with the pores, was visible.

It is difficult to say to what this high resistance of torus, pit membrane and border is due, but the yellow appearance of torus and membrane and the complete lack of color of the border suggest that the two former are lignin

**Additional "soluble lignin" probably remains in solution.*

and the last is cellulose. In sections treated under our conditions, residual lignin is yellowish and cellulose quite uncolored. The difficulties encountered by Scarth and Spier (57) when they tried to loosen the tori of red spruce heartwood are perhaps rendered more intelligible by these observations, while the high resistance of the medullary ray cells is in accord with the findings of Harlow and Wise (25), that in the rays of *Quercus alba* and *Casuarina inophloia*, which were chosen for the large size of their rays, the lignin is higher and the cellulose lower than in the wood as a whole.

The fact that lactic acid-hydrochloric acid mixture extracts 42% of the wall indicates that substances other than lignin are removed, and this is probably due to hydrolysis of hemicelluloses, etc. Lactic acid alone is not an effective solvent for lignin, and the necessary use of strongly acid mixtures rather removes it from the class of mild solvents. In fact, it is clear that none of the solvents tried is really specific for lignin and it is this that renders this type of work highly unsatisfactory.

Biological analysis of the cell wall. The employment of enzymes to bring about separation of the cell wall constituents has already received considerable attention at the hands of a number of workers.

R. Hartig (26) in 1878 published a volume on decay of woods, and Czapek (14) described as "hadromase" an enzyme capable of destroying lignin. Further investigations of the enzymes of fungi were made by Kohnstamm (33), Buller (8) and Reed (48). Much of this early work is reviewed by Potter (46), while papers by Zeller (69, 70) deal with the physiology of wood-destroying fungi and include fairly extensive bibliographies. From *Lenzites saepiaria* Zeller described ligninase (the "hadromase" of Czapek), cellulase, hemicellulase, pectase and pectinase, as well as many other enzymes acting on simple sugars, etc. The fact that cellulose reactions are given by some woods only after attack by certain fungi has been advanced as proof that lignin and cellulose are combined in the cell wall (46), but we have already seen that X-ray data are against this idea.

While wood-destroying fungi are the most obvious sources of enzymes for this work, the macerating enzymes of fruits, the hemicellulases of the date, and the extremely potent cellulases of some animals might also be considered. There is the possible advantage that in using such material more specific reactions might be obtained, but it must also be remembered that some workers have claimed that cellulases may be so specific as to act only on celluloses from some sources (69, 70).

We may notice here a few of the more recent papers on this topic. Brown (7) studied the action of *Botrytis cinerea* on non-woody tissues and found that its activity results in maceration. This suggests that its action is practically confined to the middle lamella, which presumably was composed of pectic materials in the tissues studied. Bray and Andrews (6) followed the chemical changes in groundwood during decay. They found that the brown rots have a selective action on cellulose and record that in one case 54% of the cellulose

was hydrolyzed and only 3% of lignin. Barton-Wright and Boswell (4) came to similar conclusions as a result of a study of dry-rot (*Merulius lachrymans*) which hydrolyzed mannans and galactans, then cellulose, but not lignin. Campbell and Booth (9) remark that "decay of brown-rot type should be regarded as an acid hydrolysis", while Hawley, Fleck and Richards (27) state that though brown-rots (such as *Lenzites striata*, etc.) attack cellulose, the white-rots (*Polystictus hirsutus*, *Trametes pini*, etc.) preferentially destroy lignin. They found, however, that in the early stages *Polystictus* may also attack cellulose. Pentosans, apparently, were more readily used than hexosans, while Rege (49) also found that the pentosans are the most important. Certain thermophilous bacteria are destroyers of cellulose, and Viljoen, Fred and Peterson (66) describe a form that can hydrolyze cellulose rapidly at 65° C., at which temperature the action of lignin-destroying enzymes might perhaps be inhibited. In spite of all this work, however, enzymes have not yet been isolated in such a form that cellulose and lignin can be separately and quantitatively removed from woody tissue.

Apart altogether from the chemistry of the wall itself there is a further point which requires more thorough investigation and that is the transformation from sapwood to heartwood.

The permeabilities to water of heartwood and sapwood are widely different, the latter being many times more permeable than the former, and the difference usually is due either to pit closure (most softwoods) or to the formation of tyloses (hardwoods) in the cells of the heartwood, the changes being accompanied by the death of the living elements of the wood. We shall see in a later paper that the heartwood may have little or no free water in the cell lumina, while neighboring cells of the sapwood may be full of water.* No one as yet has explained adequately just how this drying may occur, though Scarth (personal communication) suggests that the water is removed by tension from above after the area to become heartwood has been cut off from supplies from below. We have already noted the resistance to solution of the bordered pits and the difficulties encountered by Scarth and Spier in their attempts to open pits, and we must admit that the nature of the material causing pit closure has not so far been established.

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**Death of diseased trees may be due to the premature formation of "heartwood" through abnormal tylose formation (35), or to closure of pits (Nelson and Beal (40) and private note to Scarth). In both cases water is unable to pass the abnormal areas.*

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STUDIES OF WOOD

II. ON THE WATER CONTENT OF CERTAIN CANADIAN TREES AND ON CHANGES IN THE WATER-GAS SYSTEM DURING SEASONING AND FLOTATION[1]

By R. Darnley Gibbs[2]

Abstract

The chief species studied were paper birch, poplar (*Populus tremuloides*), jack pine, white spruce, and balsam fir.

Methods for the study of water contents are described. Determinations of densities and swelling percentages are summarized. Conversion factors that may be employed to convert moisture contents based on dry weight into percentages of original volume are:—for jack pine 0.38, for balsam 0.315, for poplar 0.42 and for birch 0.49.

The hardwoods examined show a maximum water content in spring and a sharp drop in the summer. This appears to vary from year to year and the possible reasons for this variation are discussed. In 1931 birch and poplar lost half their total (spring) water during the summer months. In birch this may not be made up until the following spring. The softwoods show no marked seasonal changes in water content.

The distribution of water is characteristic for each species. Changes in distribution throughout the year have been followed. In birch all parts of the wood (there is no heartwood) join in the seasonal changes; in poplar only the sapwood varies in water content. The results of individual year-ring analyses and of borings at different heights point to uniform water content in corresponding parts of the tree.

Diurnal changes in water content have been investigated and rapid fluctuations recorded. These point to a decrease during the morning followed by an increase later in the day. These variations are correlated with tension changes and no doubt also with transpiration. It seems certain that the actual amount of gas in the tree varies but little during the diurnal changes, though it does vary with the seasonal fluctuations in water content.

Girdling of birch, balsam and spruce is described and the effects on water contents are followed. It is shown that in the case of birch, removal of wood to a depth of more than one inch leads to little change during two seasons. This is correlated with the continued activity of all parts of the wood. In balsam, almost complete drying of the sapwood within two or three months follows girdling through the sapwood. The characteristic wet patches of balsam heartwood, however, are unaffected, and it is concluded that these have no connection with the sapwood and so play no part in water conduction. The results from spruce are irregular.

Experiments on seasoning and flotation in the field and in the laboratory are described. The summer seasoning of "sour-felled" birch is more rapid than that of normal or of peeled logs or the normal water loss of living standing trees, and this must be due to evaporation from the leaves.

The effects of log length, of barking, and of end and/or side painting on rate of penetration of water have been investigated. While penetration of water is chiefly through the ends of logs, escape of dissolved air is largely in the radial direction, and so end penetration is less important than might be expected. There is considerable top drying from unseasoned floating logs (in laboratory tanks), which may assist in solution and removal of air and so hasten rather than slow up sinkage of the log. Seasoning followed by end painting results in very slow entry of water and so is excellent in flotation.

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Contribution from the Department of Botany, McGill University, with financial assistance from the National Research Council of Canada. From a thesis approved for the degree of Doctor of Philosophy in the University of London, England.
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Introduction

This is the second of a series of three papers under the general title of "Studies of Wood". The first (12) dealt with work on the structure of the cell wall. The present paper approaches the subject from an entirely different viewpoint.

The density of actual wood substance is about 1.52–1.56. It is therefore denser than water and wood floats only because of entrapped air. It is well known that the natural gas content of wood varies with the species considered and with the season.

In eastern Canada, logs are floated from wood to mill, and when gas content is low, or when the "drive" is long, buoyancy may be lost and the logs sink. The following studies were carried out with a view to determining (*a*) the season at which trees are driest, and (*b*) the changes in water content caused by girdling, by seasoning, and by flotation. The hardwoods—birch and poplar—are notoriously poor floaters, so that attention has been concentrated on these. At present they are often left standing because flotation is so uncertain.

Acknowledgment of help received in this work has been made in the first paper of the series.

The following trees were studied:—

paper birch (*Betula alba* var. *papyrifera* (Marsh) Spach.), poplar (*Populus tremuloides* Michx.), jack pine (*Pinus banksiana* Lamb), white spruce (*Picea canadensis* (Mill.) P.S.P.), balsam fir (*Abies balsamea* (L) Mill.);

and to a lesser extent:—

sugar maple (*Acer saccharum* Marsh), tamarack (*Larix laricina* (DuRoi) Koch), yellow birch (*Betula lutea* Michx. f.).

In earlier work it was decided that for certain purposes the selection of groups of three trees, and the averaging of results from each group, gave sufficiently trustworthy results. From each tree, sets of samples out of top, middle and butt logs were taken, the nine sets of samples so obtained being referred to as "standard lots". "Butt" samples were taken about three feet from the ground, "middle" samples from the unbranched trunk at about 25 ft., and "top" samples at 40–50 ft., in the crown of the tree. For the most part, two discs were taken from each region, one being used as a whole, the other being chipped into smaller sections, very much as was Craib's (6, 7, 8) material. Two objections to these methods have been advanced. In the first place, they involve destroying the tree, which limits its use to a single experiment; second, there is a possibility (if high tension exists in the tree) of marked changes in water distribution during cutting. Objections have been met as follows:—

a. A borer on the lines of the common "increment" borer, but giving cylinders of wood $\frac{5}{16}$ in. in diameter, was used for several series of experiments.

b. Punchings were made, with a hollow punch giving a cylinder ½ in. in diameter. This was placed against the tree after removal of the outer bark and driven home by a single smart blow from a heavy hammer. There was no chance in this case for migration of water on release of tension, except in a radial direction, and this could not occur in the second or two elapsing before removal of the punching. This method could be used only for the outer half-inch or so of the wood, but it is in that region that higher tensions and possible migrations are to be expected.

The results from a series of experiments carried out on a hot dry day, when tensions were high, were so nearly uniform that it was concluded that any or all of these methods might be employed with reasonable confidence.

It is well, perhaps, to explain more fully the exact technique involved in these methods. Craib (6, 7, 8) and Robert Hartig (15, 16, 17) employed the disc method, the former in his later work taking four strips from outside to centre. Birch, jack pine and spruce have been found so regular in their water distribution, however, that a single transverse strip divided into nine pieces (1 and 9, 2 and 8, 3 and 7, and 4 and 6 being averaged) has been used. In all but the birch the samples were of such a size that 1, 2 and 3, and 7, 8 and 9 represent sapwood, the others being heartwood. Poplar and balsam have much more irregular water content, but the errors due to the strip method, as against division of the whole disc are comparatively small. The blocks, as removed from the strip, are rapidly trimmed on all sides (to eliminate errors due to surface drying) and weighed at once in the field. Borings and punchings are placed at once in weighed rubber-stoppered bottles and weighed at leisure. In all cases the first weighing is recorded as "wet weight"; the samples are dried for 36–48 hr. at 100 to 105° C. in an electric oven, cooled in a desiccator and weighed as rapidly as possible for "dry weight".

Distribution of Wood, Water and Gas in the Tree

The walls of wood in the natural state are saturated with water, since *free* water is always present somewhere in the tree and the imbibition pressure of the cell wall is very high. Several authors—Robert Hartig (15–17), Dunlap (9), Pidgeon (24), Grace and Maass (14), Wilson (36), Sachs (26)—have attempted to determine this water.*

It is doubtful whether the true values for the water held by the wood itself can be determined, as condensation takes place in the lumina of the cells, but by extrapolation of adsorption and desorption curves, Pidgeon and Grace and Maass obtain comparative values which probably approach them very closely. These authors give a higher value for pine than for spruce, while Robert Hartig, using different species, found the value for pine consider-

* *Since writing the above our attention has been called to the work of Beversluis (Mededeel. Landbouwhoogeschool Wageningen 35 : 3-31, 1931. English summary). As a result of shrinkage studies he concludes that ". . . , fibre saturation-point, therefore, does not correspond with a moisture content of the wood of 25–30%, as is generally stated, but with one of 15–16% (of oven-dry weight)".*

ably lower than that for spruce (Table I). Barkas (1), using an optical method for the determination of adsorption of water by wood, records a value of 20% for adsorption on Sitka spruce flour, indicating that non-capillary forces hold most of the water of saturation.

TABLE I

SATURATION VALUES AND NATURAL WATER CONTENTS OF CERTAIN WOODS
(Values expressed as % based on dry weight)

Species	Saturation value, etc.	Natural water content (average throughout the year)
Pinus sylvestris *P. banksiana*	Hartig 30 (heartwood) Sachs 31 Pidgeon 31 (sapwood—absorption)	Hartig 31 (heartwood) Gibbs 33 (heartwood)
Picea excelsa *P. canadensis*	Hartig 37 (heartwood) Grace and Maass 28 (heartwood—desorption) Pidgeon 24 (sapwood—absorption)	Hartig 37 (heartwood) Gibbs 40 (heartwood)
Betula verrucosa *B. papyrifera* *B. lutea*	Hartig 42 (heartwood ?) Grace and Maass 27 (desorption ?) Wilson 27, 29 (used two different methods)	
Average of seven American species	Dunlap 33 (about)	

In the standing tree, while free water is always present in the sapwood, there may or may not be free water in the heartwood. The remarkably constant figures obtained for water in jack pine and spruce heartwoods suggest that in these cases no free water is present. Robert Hartig thought this to be the case (Table I). The values given for natural water content of heartwood are averages of large numbers of determinations and include results from samples which, without doubt, had a little free water, so they are probably slightly higher than the true values for wall saturation.

The free water which is usually present in the cell cavities of the sapwood is rarely sufficient to fill all the lumina, the remaining spaces containing a gas mixture of variable composition. Thus, in order to have a clear conception of wood, it is necessary to know the volume and density of wall material, the volume of water, the volume and nature of the gas, the distribution of wood, water and gas in the sample, how this last changes in the living tree, and how, and at what rate, it can be changed by seasoning and flotation. The necessary procedure has been described in earlier papers (11, 13) and need not be detailed here.

If measurements of appropriate series of blocks be made, the variation in gross composition throughout the tree at various seasons may be determined. The distribution of wall substance will not vary from season to season and, when once investigated, may be taken for granted, so that from measure-

ment of water content we may obtain the gas content by difference (*i.e.*, gas = 100 − (wood + water)). In practice it is found that the percentage of space occupied by cell wall in a block of wood varies but slightly for a given

TABLE II

AMOUNT OF CELL WALL, DENSITY AND SWELLING OF CERTAIN WOODS

Species	Position in tree	% wood (based on fresh vol.)	Density (water = 1.0)	Swelling (% of dry vol.)	Remarks
Pinus banksiana	Centre of heartwood	23 −	0.39	8	Average of four logs
	Outer sapwood	25 +	0.45	12	
Abies balsamea	Sapwood:— outer	19	0.34	11	Average of top, middle and butt logs from each of three trees. (See also Fig. 1).
	mid.	19	0.34	12	
	inner	20	0.35	12	
	Heartwood:— outer	20	0.35	10	
	centre	22	0.38	9	
Betula papyrifera		Average 33. Usually lowest at centre, highest at outside. Usually higher in slowly grown logs	Average 0.6. Usually denser toward outside of log. Higher in slowly grown logs	17 to 24 Average 21	Average of 14 logs. (See Gibbs (11, p. 432) and Fig. 5). Results variable; not correlated with position
Populus tremuloides		Average 27. (20–25 in a log with 6 rings to the inch; 27–30 in a log with 19 rings to the inch)	Ranged from 0.37 to 0.55. Average 0.47	12	Average of 4 logs. Results very variable

species and may be taken as constant for all but finer work. The slight variations found are perfectly definite, however, and to a certain extent characteristic for some trees. The results of the present studies and of those of R. Hartig (15–17) are summarized in Table II and in Figs. 1, 2 and 3.

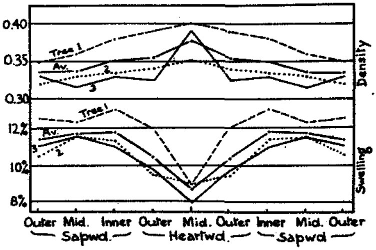

FIG. 1. *Abies balsamea. Density and swelling. Each curve represents average of top, middle and butt logs.*

Reference to Hartig's results (Figs. 2 and 3) show that while the sapwoods of

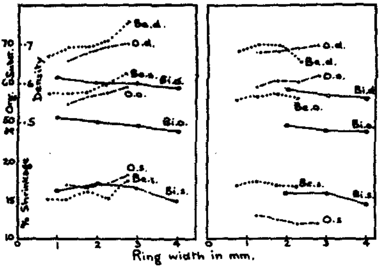

FIG. 2. *Density, wall substance and shrinkage of certain woods as related to ring width (from R. Hartig's figures). Hardwoods (sapwood left, heartwood right):—d = density (water = 1). o = organic substance (gm. in 100 cc. fresh wood). s = shrinkage (% of fresh volume). Be = beech (Fagus sylvatica). Bi = birch (Betula verrucosa). O = oak (Quercus pedunculata).*

larch, oak and beech showed increasing density, organic substance (the equivalent of "wood" in Table II) and swelling with increasing ring width, the remaining woods examined all showed exactly the reverse relations. These differences are probably due to variations in the relative proportions of spring and summer woods in the tree (4). Where wide rings are due to continued formation of summer wood, density should increase with ring width, and *vice versa*. The variations observed are of some importance when we wish to calculate the proportions of water, "wood" and gas in a given sample. If we take average figures for density, "wood" and swelling for each species and apply these to any particular sample, the error involved is considerable but calculations of water, "wood" and gas from the easily obtained wet and dry weights of a piece of wood are sufficiently accurate for comparative purposes. The "conversion factor" for converting "% water based on dry weight" into "% water based on original fresh volume" is $\frac{\text{density} \times \text{dry volume}}{\text{wet volume}}$ (see Gibbs (11)). These factors for the trees examined work out at 0.38 for jack pine, 0.315 for balsam, 0.42 for poplar, and 0.49 for birch.

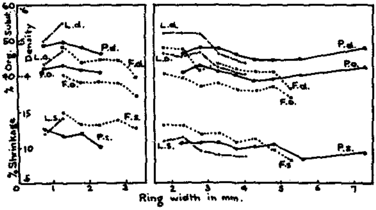

FIG. 3. *Density, wall substance and shrinkage of certain woods as related to ring width (from R. Hartig's figures). Softwoods:—L = larch (Larix europaea). P = pine (Pinus sylvestris). F = fir (Picea excelsa).*

Seasonal Changes in Water Content

The trees used came from three stations:—

(*a*) near Lac Onatchiway, about forty miles north of Chicoutimi,

(*b*) near Ste. Anne de Bellevue, twenty miles from Montreal, and

(*c*) from Windsor Mills, P.Q. (yellow birch only).

As the first station is three hundred miles from Montreal, earlier material was sent in by express and the consequent delay in handling led to errors which were evaluated by a series of tests; birch, spruce and poplar logs being sampled in the bush and as received at the laboratory at Montreal. The changes in the water content of spruce and birch were found to be slight, that in poplar was somewhat greater. All later work was carried out in the field.

1. Paper Birch (*Betula alba* var. *papyrifera*)

(a) *Strip and Disc Method. Lac Onatchiway Material (Table III)*

The water content of the tree as a whole fluctuates during the year, the maximum occurring just before leaf opening (end of May), the minimum in July and August (Fig. 4). In 1930 the minimum was just over 80% (based on dry weight). In 1929 it was probably lower than that (though higher, for the reason stated, than the figure indicated, 54%), while in 1931 it was 54%. This value was recorded in July and again in August.

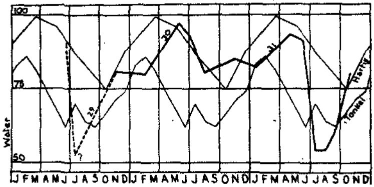

Fig. 4. *Birch. Change in water content of whole tree throughout the years 1929 to 1931. The results of Hartig (15, 16) and Tonkel (quoted in 4) are included. They represent results for a single year, but are repeated for purposes of comparison.*

It is quite possible that the maximum has been missed, for calculations from volume and density measurements indicate that the wood of the trees investigated could hold at saturation about 135% of water. This would correspond to a density of 1.16 and gas content of 0%. In wood with red heart, figures in excess of 130% (*i.e.*, about the possible maximum) have been noted, but the highest water content of a tree, as a whole, that has been encountered was 103% in early June, 1930. This corresponds to a density of 1.00 and gas content of about 22% of the volume. Merwin and Lyon (23) have reported figures suggesting approximation to saturation during sap flow. It might be thought that the water content of the tree during the period when sap flows would remain more or less constant, but a fall in water content of standing trees at a time when stumps of cut trees continue to bleed has been noted.

It is, of course, no mere coincidence that the maximum water content is just before leaf opening and the minimum just before leaf fall, but this point will be discussed in detail in the third paper of this series.

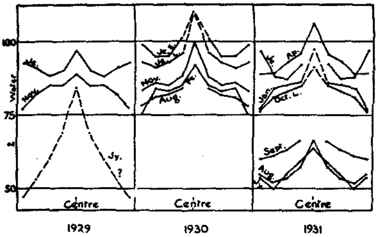

FIG. 5. *Birch. Changes in distribution of water across tree during years 1929 to 1931. Most curves represent averages of nine logs from three trees (Table III). Je. E.—early June. Je. L.—late June. Oct. L.—late Oct. Curves are dotted where unreliable owing to presence of red heart or other causes (see text)*

So far, only the total amount of water in the trees has been considered; there remains the question of lateral and longitudinal distribution. This is indicated by Fig. 5, and Table III. It will be seen that, while the top logs are usually a little drier than the butt logs, the differences are slight and somewhat irregular (see below for further comment on this point).

TABLE III

SEASONAL CHANGES IN WATER CONTENT OF BIRCH

(Disc and strip method)

Date	Remarks	Position in tree	Water (as % dry weight) Outside to centre					Water in disc (calc.)
1929								
June	Samples analyzed in lab.	Average	93	91	88	90	97	91
July	Samples analyzed in lab. Some delay. Ten trees.	Average	47	53	60	69	84	54
Nov.	Considerable delay in sampling. Four trees.	Average	77	82	85	85	89	81
1930								
Feb.	Sampled at lab. Short delay. Three trees.	Tops	66	82	82	83	89	76
		Middles	78	89	84	85	95	83
		Butts	80	82	84	86	—	82
		Average	75	84	84	88	(92)	80
Early June	Sampled at lab. Wrapped for shipment. Three trees.	Tops	97	96	97	97	100	97
		Middles	95	93	93	97	101	94
		Butts	105	95	95	108	130	100
		Average	99	95	95	101	(110)	97
June 24	Sampled when felled. Three trees.	Average	93	91	93	96	(110)	93
Aug.	Sampled at lab. Wrapped for shipment. Three trees.	Average	78	81	82	85	90	80
Oct.	Sampled in bush. One tree.	Average	91	82	80	81	88	85

TABLE III—*Concluded*

SEASONAL CHANGES IN WATER CONTENT OF BIRCH

(Disc and strip method)

Date	Remarks	Position in tree	Water (as % dry weight)					Water in disc (calc.)
			Outside to centre					
1930								
Nov.	Samples wrapped for shipment. Three trees.	Tops	81	89	89	91	94	86
		Middles	83	85	83	88	103	84
		Butts	86	83	83	86	—	85
		Average	83	86	85	88	(98)	85
1931								
Jan.	Sampled in bush. Three trees.	Tops	71	82	85	87	88	79
		Middles	79	86	87	88	97	84
		Butts	82	84	83	84	**108**	83
		Average	77	84	85	86	(97)	82
Late April	Sampled in bush. Three trees.	Tops	59	81	98	102	106	78
		Middles	103	93	94	96	**111**	98
		Butts	105	91	85	93	**108**	96
		Average	89	88	92	97	**108**	91
Early June	Sampled in bush. Three trees.	Tops	80	80	80	83	93	80
		Middles	107	93	90	93	104	98
		Butts	105	94	90	99	**128**	98
		Average	97	89	87	92	(108)	92
July	Sampled in bush. Three trees.	Tops	58	50	53	57	63	57
		Middles	52	48	53	58	68	52
		Butts	53	52	55	62	62	54
		Average	54	50	54	59	64	54
Aug.	Sampled in bush. Three trees.	Tops	59	55	56	58	66	57
		Middles	52	51	54	58	64	53
		Butts	55	51	52	58	67	54
		Average	55	52	54	58	66	54
Sept.	Sampled in bush. Three trees.	Tops	54	55	57	57	67	55
		Middles	57	59	60	63	72	59
		Butts	69	70	72	77	**82**	71
		Average	60	61	63	66	(74)	62
Late Oct.	Sampled in bush. Three trees.	Tops	70	83	87	86	89	79
		Middles	83	84	85	86	(91)	84
		Butts	78	77	78	83	(93)	78
		Average	77	81	83	85	(91)	80

Figures in bold face include red heart. Averages bracketed are incomplete.

There is no indication of heartwood and sapwood in paper birch but the greater variations in water content of the younger wood seem to indicate that it serves more effectively as a water channel than do the inner parts of the tree. Scarth (unpublished work), using similar trees, found that dyes on entering borings rose more rapidly in the outer rings.

The work of MacDougal, Overton and Smith (22) on distribution of gas in the tree suggested an examination for variations from ring to ring. Quantitative determinations of water content in a single ring proved difficult but not impossible. Results are drawn from the following material:—

October, 1930.

Birch—Top, middle and butt logs from a single tree.

Poplar and spruce—Top, middle and butt logs from each of three trees.

January, 1931.

Birch, poplar, balsam and larch—Top, middle and butt logs from each of three trees.

Spruce—Top, middle and butt logs from each of two trees.

July, 1931.

Birch, poplar and spruce—Top, middle and butt logs from each of three trees.

Balsam—Top, middle and butt logs from each of two trees.

The complete series is summarized in Fig. 6, the curves representing averages of samples from top, middle and butt logs.

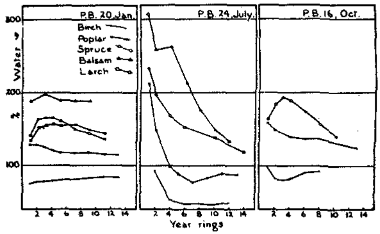

FIG. 6. *Water content of outer rings at different seasons. The water content is expressed as percentage of dry weight. The numbering of rings is from the outside.*

The change in form of the curve for water content is really less striking than appears from Fig. 6, for it must be remembered that Ring 1 of the July samples is the newly formed ring, absent from those of January. It has a very high water content in spruce, poplar and balsam, and no doubt in birch also, but in the last it was not possible to separate it from the other rings. The changes in the other rings are not great, but there are indications of some seasonal changes in the softwoods. This is of interest since gross analyses do not bring these out (see below). Those of poplar and birch show decreased water content in July, corresponding to the general decrease in the tree as a whole. The ring for the current year contains less water in October than in July, which is to be expected.

(*b*) *Boring Method. Lac Onatchiway and Ste. Anne's Material*

Results are summarized in Fig. 7. The fall in water content as indicated by figures from cut trees is demonstrated even more effectively here. It is interesting to note that the smaller birch at Ste. Anne's shows a fall to a lower value than the larger trees near Lac Onatchiway. The much later leaf opening and earlier leaf fall at the latter station (which is further north and at an elevation of about 1,000 ft.) also are reflected in the figures. When borings were taken in October 1931 at Lac Onatchiway bleeding was noted. This is rather unusual at that season, but was somewhat less surprising in view of the spring-like weather which prevailed at the time.*

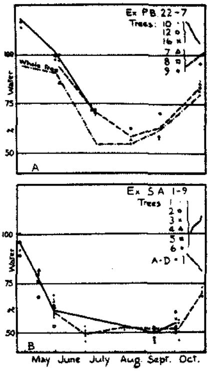

FIG. 7. *Birch. Decrease in water content during the summer of 1931, as shown by borings to a depth of one inch.*
A. *On the Price Brothers Limits, two sets of trees D.B.H. 8 in. The water content of whole trees, as determined by the disc method, is added for comparison.*
B. *Near Ste. Anne de Bellevue, two sets of trees D.B.H. 4 to 5 in.*

2. YELLOW BIRCH (*Betula lutea*)

A few figures from *Betula lutea* indicate a comparable drop in water content during the summer months. Two large trees (ca. 18 in. D.B.H.) at Windsor Mills, P.Q., were bored to a depth of one inch on June 3, 1932. The moisture contents of the borings were 94 and 84% respectively, while in early September they were 61 and 50%.

3. POPLAR (*Populus tremuloides*)

(*a*) *Strip and Disc Method. Lac Onatchiway Material. (Figs. 8 and 9, and Table IV.)*

TABLE IV

SEASONAL CHANGES IN WATER CONTENT OF POPLAR

Date	Remarks	Position in tree	Water (as % dry weight) Sapwood Outer	Sapwood Mid.	Sapwood Inner	Heartwood Outer	Heartwood Centre	Water in disc (calc.)
1930 Early Feb.	Sampled at lab. Short delay. Three trees.	Tops	147	125	100	65	60	123
		Middles	156	123	93	55	62	123
		Butts	162	130	102	74	78	132
		Average	155	126	98	65	67	126

* *Dr. C. W. Townsend on October 6, 1927, found sap flowing from fresh holes made by a yellow-bellied sapsucker in an apple tree (personal note to Professor V. C. Wynne-Edwards). Autumnal bleeding is probably more common than is usually supposed.*

TABLE IV—*Concluded*

SEASONAL CHANGES IN WATER CONTENT OF POPLAR

Date	Remarks	Position in tree	Water (as % dry weight)					Water in disc (calc.)
			Sapwood			Heartwood		
			Outer	Mid.	Inner	Outer	Centre	
1930								
Late April	Sampled at lab. Short delay. Three trees.	Tops	116	106	89	79	75	104
		Middles	131	111	93	72	62	112
		Butts	131	127	128	72	56	123
		Average	126	115	103	74	64	113
Mid-Aug.	Sampled at lab. Short delay. Three trees.	Tops	100	94	78	70	79	91
		Middles	84	83	77	69	67	81
		Butts	78	75	92	**97**	**112**	82
		Average	87	84	82	(79)	(89)	84
Late Oct.	Sampled in bush. Three trees.	Tops	132	115	84	79	66	112
		Middles	154	132	105	80	77	130
		Butts	125	96	81	71	74	102
		Average	137	114	90	77	72	115
Mid-Dec.	Sampled at lab. Very short delay. Three trees.	Tops	151	128	108	**91**	53	130
		Middles	160	135	106	59	61	132
		Butts	156	146	136	97	65	143
		Average	156	136	117	(82)	60	135
1931								
Jan.	Standard lot of three trees. Sampled in bush.	Tops	134	122	104	69	63	118
		Middles	123	114	104	60	64	110
		Butts	115	104	101	73	**96**	105
		Average	124	113	103	67	(74)	111
Late April	Standard lot of three trees. Sampled in bush.	Tops	136	118	101	54	54	115
		Middles	143	126	108	73	70	124
		Butts	163	128	100	**107**	**116**	134
		Average	147	124	103	(78)	(80)	124
June	Standard lot of three trees. Sampled in bush.	Tops	105	99	88	60	68	95
		Middles	88	78	80	62	69	80
		Butts	86	76	81	111	122	85
		Average	93	84	83	(78)	(86)	87
Aug.	Standard lot of three trees. Sampled in bush.	Tops	96	87	81	62	57	87
		Middles	79	76	78	57	49	76
		Butts	83	73	72	**92**	**81**	79
		Average	86	79	77	(70)	(63)	81
Sept.	Standard lot of three trees. Sampled in bush.	Tops	80	76	70	61	57	75
		Middles	67	66	66	66	64	66
		Butts	61	60	63	—	—	61
		Average	69	67	66	(64)	(61)	68
Late Oct.	Standard lot of three trees. Sampled in bush.	Tops	131	116	96	(46)	59	111
		Middles	105	92	93	57	62	94
		Butts	92	93	106	**91**	**78**	95
		Average	109	100	98	(65)	(66)	100

Figures in bold face are from blocks with rot; figures bracketed are averages including such values or are from incomplete records.

The water content of the tree as a whole varies very much as does that of birch, a decided drop in water content occurring during the summer months. Certain differences, however, are apparent. The percentage of water present is decidedly higher, ranging from about 130 in December to 65 in September (the maxima and minima for birch are about 100 and 50), and the maximum water content may be in December rather than at leaf opening. Poplar is more variable than birch and one must be conservative in estimates of the reliability of figures. Thus, in December 1930, a water content of 129% was recorded, as against 109% and 117% in January and April, 1931. It is possible that this lower value in January *really* records loss of water from the bark during the time when the soil is frozen and root activity (presumably) is completely at a standstill, and the higher figure for April may correspond to the sap-flow maximum of birch. Poplar logs lose water much more rapidly than birch or spruce, and the loss by evaporation from the leafless tree may also be relatively great. Experimental evidence for this is given in a later paper. Tonkel (see Fig. 8), working in Russia, though finding a continued rise from September to February, recorded a slight fall as early as March—some time before root activity could have commenced in such a rigorous climate.

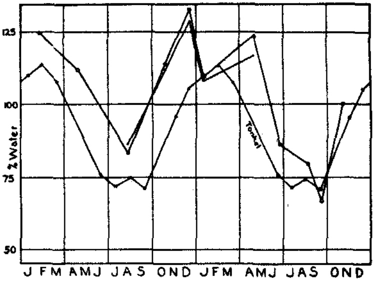

FIG. 8. *Poplar. Changes in water content of whole tree throughout the year. The line with crosses is from Tonkel (in Büsgen (4)). The line with dots joins values obtained from discs; the line with circles, values obtained by calculation from those of small blocks. Tonkel's curve is repeated for comparison. The other curves are from records for February 1930 to October 1931 (Table IV).*

The very different summer values obtained for birch in 1930 and 1931 are again met with here. The lowest figure recorded for poplar in 1930 was 86 (in August), and even this was lower than the actual value owing to loss in transit, while the results for June, August and September of 1931 are about 85, 80 and 65 respectively. The September figure for poplar is considerably below the August value: in birch the water content is rising by September. This is correlated with the fact that birch was losing its leaves during the latter month, while those of poplar remained quite green and functional. In both species, however, there is a marked upswing in October, following complete defoliation.

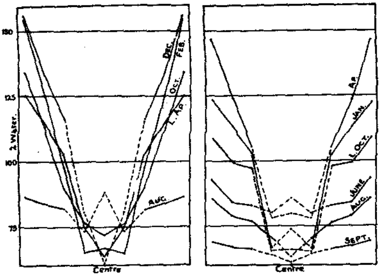

FIG. 9. *Poplar. Changes in distribution of water across the tree during 1930 (left) and January–October 1931. Curves are dotted where unreliable owing to presence of fungus infection.*

Distribution in the tree is very different from that of birch (compare Figs. 5 and 9). This, of course, is due to the formation of heartwood in poplar. The heartwood occupies only a relatively small proportion of the volume of the tree which, from the viewpoint of flotation, is unfortunate, as it is fairly and more or less uniformly dry. In larger trees, fungus infection is very common indeed, and infected wood is much wetter.

The sapwood throughout the winter is very wet. The maximum possible water content of poplar is about 170% (based on dry weight) and in December and February of 1930 over 150% of water in the outer sapwood (after some loss during transit) was recorded. As one passes inward a steady decrease in water is noted, but the value rarely falls below 100% in the winter months. Throughout the summer a very marked decrease in water content of sapwood occurs and this was particularly marked in 1931, dropping to about half the winter value. For water contents of individual rings see Fig. 6.

(*b*) *Boring Method* (*Lac Onatchiway and Ste. Anne's Material*)

Results are summarized in Fig. 10.

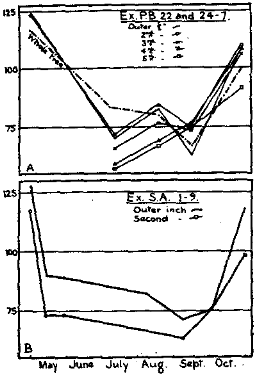

FIG. 10. *Poplar. Decrease in water content during the summer of 1931, as shown by borings.*

A. *On the Price Brothers limits, trees 8 in. D.B.H.; borings to a depth of two and a half inches. Water content of whole tree by disc method added for comparison.*

B. *Near Ste. Anne de Bellevue. Borings to a depth of two inches. Trees 8 to 15 in. D.B.H.*

4. JACK PINE (*Pinus banksiana*)

The results from jack pine are so uniform as to need very little discussion here. The heartwood seems never to contain free water. The water present, which is sufficient only to saturate the cell walls, averages about 34%. The sapwood is always wet and there is no evidence of

any marked seasonal variation (but see individual growth ring analyses). This is contrary to the findings of Hartig (15-17) for ***Pinus sylvestris*** in Europe. Table V gives a very brief summary of the results.

TABLE V

WATER CONTENT OF JACK PINE

Date	Position in tree	Water content					Remarks
		Sapwood			Heartwood		
		Outer	Mid.	Inner	Outer	Centre	
1929							
Mar.	Average of all pieces	139	—	144	32	37	Analyzed after some delay.
July	Average of all pieces	148	—	132	30	32	Analyzed after some delay
Aug.	Average of all pieces	141	124	103	33	32	Analyzed after some delay
Nov.	Average of all pieces	160	—	147	33	34	Analyzed after some delay
1930							
Feb.	Average of all pieces	161	—	148	32	33	Analyzed in bush
Dec.	Average of all pieces	138	135	131	35	34	Analyzed in bush
1931							
Sept.	Tops	179	170	179	38	36	Three trees.
	Middles	173	177	174	34	35	Analyzed in
	Butts	164	164	164	35	35	bush
	Average	172	170	172	36	35	

There is very little difference in water content between the sapwoods of top, middle and butt logs. Top logs, of course, are wetter as a whole because of the higher proportion of sapwood.

The maximum possible water content of jack pine is about 205% (based on dry weight) and values approximating this have been obtained in outer sapwood.

5. SPRUCE (*Picea canadensis*)

The results are summarized in Table VI. As in jack pine, there is no appreciable change in water content throughout the year. The upper parts of the tree seem to be a little wetter than the lower parts (quite apart from the question of proportions of heartwood and sapwood). The amount of water in the heartwood is remarkably constant, but probably just sufficient to saturate the cell walls, as in jack pine. For the distribution of water in the outer growth rings see Fig. 6 and below.

TABLE VI

WATER CONTENT OF SPRUCE

Date	Position in tree	Water content					Remarks
		Sapwood			Heartwood		
		Outer	Mid.	Inner	Outer	Centre	
1930							
Feb.	Average of all pieces	160	—	115	42	44	Slight delay in analysis
Aug.	Average of all pieces	—	171	—	40		
Oct.	Average of all pieces	181	177	137	42	48	Slight delay in analysis
1931							
Jan.	Tops	166	170	139	42	46	Three trees. Analyzed in bush
	Middles	159	160	138	43	40	
	Butts	146	146	127	41	40	
	Average	157	159	135	42	42	
July	Tops	182	146	132	43	41	Three trees. Analyzed in bush
	Middles	174	154	131	39	45	
	Butts	161	122	112	35	38	
	Average	172	141	125	39	41	
Aug.	Tops	192	166	144	—	39	Three trees. Analyzed in bush
	Middles	179	156	120	39	42	
	Butts	165	146	124	37	48	
	Average	179	156	129	38	43	
Sept.	Tops	198	178	144	44	44	Three trees. Analyzed in bush
	Middles	181	162	141	41	40	
	Butts	187	170	148	35	40	
	Average	189	170	144	40	41	

6. Balsam (*Abies balsamea*)

The results are summarized in Table VII. Balsam, of the trees investigated, has the highest water content, averaging well over 200% in the sapwood as a whole. The average maximum possible water content of this tree is about 260%. Actually, figures in excess of 300% have been recorded and the figure of 260% is commonly met with. The gas content of the sapwood of balsam, therefore, is very low indeed.

The heartwood of this tree presents a curious problem. It is never (?) uniformly dry, but has irregular wet patches which are commonly associated with "blind knots" and small branches but do not follow these exclusively. The line of demarcation between wet and dry wood seems almost invariably to be very sharp and connection between these wet zones and the sapwood has not been observed. On the contrary, a complete, relatively dry ring usually is evident between the two. The water content of the driest heartwood probably represents water of wall saturation, while the water content of the wet patches is about that of the sapwood, and it would be natural to

TABLE VII

WATER CONTENT OF BALSAM

Date	Position in tree	Water content					Remarks
		Sapwood			Heartwood		
		Outer	Mid.	Inner	Driest	Wettest	
1930							
June	Average of all pieces	—	197*	—			Slight delay in sampling
Nov.	Average of all pieces	—	213*	—			Slight delay in sampling
Dec.	Average of all pieces	222	213	191			Slight delay in sampling
1931							
	Tops	186	179	182	77	209	Three trees. Sampled in bush
Jan.	Middles	215	213	196	47	220	
	Butts	189	199	185	43	253	
	Average	197	197	188	—	—	
July	Tops	264	208	184	63	88	Two trees. Sampled in bush
	Middles	269	210	174	60	168	
	Butts	252	206	184	38	239	
	Average	262	208	181	—	—	
Aug.	Tops	257	224	186	—	—	Two trees. Sampled in bush
	Middles	271	237	209	32	—	
	Butts	268	241	196	41	—	
	Average	265	234	197	—	—	
Sept.	Tops	223	215	189	116	180	Three trees. Sampled in bush
	Middles	251	229	208	42	246	
	Butts	248	211	190	42	287	
	Average	241	218	196	—	—	

**Average for all parts of sapwood.*

suppose that these regions represent continuing patches of sapwood, but the evidence from girdled trees is against this (see below). The sapwood of balsam is, as in most trees, much more permeable to water than the heartwood. The wet patches of the heartwood, while somewhat more permeable than the dry, fall far short of the sapwood in permeability. In two trees examined, absolutely no correlation between the position of these wet areas and orientation of main branches and roots could be found. In cases of fungus infection, the wetness precedes the fungus infection. It is not, as in red heart of birch, a result of it.

7. LARCH (*Larix laricina*)

The distribution of water seems to resemble that in spruce. In January 1930 three trees had an average water content in the sapwood of about 150% and in the heartwood of about 60%, while the distribution of water in the outer growth rings was almost exactly the same as in spruce (Fig. 6).

In view of the deciduous character of larch it would be interesting to follow its water content throughout the year, since it may be expected (unlike spruce, balsam and pine) to show decided seasonal changes. Th. Hartig, indeed, has recorded a summer drop in water content of *Larix europaea* in Europe and its transpiration is relatively high.

Diurnal Variations in Water Content in the Living Tree

Considerable variations of water content within twenty-four hours have been reported by several workers. Th. Hartig obtained higher figures at night and Kraus (18) recorded similar fluctuations: "Pflanzentheile sind am Tag kleiner, bei Nacht grösser . . . . Diese An- und Abschwellung resultirt aus dem periodisch schwankenden Wassergehalt der Theile; dieser ist regelmässig am Tage geringer, in der Nacht höher."

In the course of the present studies a number of observations have been made. Results obtained in 1931 are summarized in Tables VIII–XI.

TABLE VIII

BIRCH (EXPERIMENT NO. P.B. 24)

Date	July 9		July 10
Time	7:30 a.m.	11:30–12 noon	6:30–7 a.m.
Weather conditions, etc.	Fine, after fine weather.	Fine.	Dull and damp, after heavy rain.
Outer half inch	69	63 (−10)	85 (+38)
Inner half inch	68	59 (−12)	80 (+40)

Borings at breast height to a depth of 1 in. in two ½ in. sections. In this and Tables IX–XIII unbracketed figures are water contents based on dry weight, while bracketed figures represent percentage changes from the previous figures. Figures are averages from four trees.

TABLE IX

BIRCH (4–5 IN. D.B.H.) (EXPERIMENT NO. S.A. 6)

Date	August 8			August 9	
Time	9 a.m.	12 noon	6 p.m.	9 a.m.	7 p.m.
Weather conditions, etc.	Hot, fine after fine spell.	Hot and fine.	Cloudy.	Very dull.	Very dull. Fresh breeze. Some rain.
Outer inch	58	60 (+3)	60 (0)	60 (0)	62 (+4)
Inner inch	62	59 (−5)	59 (0)	60 (+2)	60 (+1)

Borings taken breast-high to depth of 2 in. in 1 in. sections. Average of four trees.

TABLE X

BIRCH (6 IN. D.B.H., HEIGHT ABOUT 50 FT.) (EXPERIMENT NO. P.B. 25)

Date	Aug. 15	Aug. 16		Aug. 17	Aug. 19
Time	3 p.m.	5:15 a.m.	6:30 p.m.	6:30 p.m.	6 a.m.
Weather conditions, etc.	Fine.	Fine.	Fine.	Fair.	(Dull on Aug. 18.) Dull.
Top	48	69 (+44)	55 (−20)	59 (+8)	70 (+18)
Butt	58	80 (+38)	66 (−17)	70 (+6)	70 (+ 1)

Bored at breast height ("butt") and about 30 ft. from the ground ("top") to a depth of one inch. Average of three trees.

TABLE XI

BIRCH (4–5 IN. D.B.H.) (EXPERIMENT NO. S.A. 7)

Date	Sept. 7			Sept. 8	
Time	9 a.m.	1 p.m.	6:15 p.m.	5 a.m.	9 a.m.
Weather conditions, etc.	Fine, sunny, cool, strong breeze.	As in the morning.	Fine.	Sunrise at 5:22 a.m. Fine.	Fine. Warmer than Sept. 7.
Outer inch	59	56 (−4)	61 (+8)	62 (+2)	59 (−5)
Inner inch	66	61 (−7)	66 (+9)	67 (+1)	62 (−7)

Bored at breast height to a depth of two inches. Average of four trees.

In the first experiment (P.B. 24, Table VIII) a substantial decrease occurred between 7:30 a.m. and noon. Heavy rain resulted in a marked upswing within the next 18 hr. In the second experiment relatively small and irregular changes were noted. In Experiment P.B. 25 (Table X) considerable fluctuations occurred. With some exceptions there were fairly close correlations between borings from breast height and from 30 ft. The last experiment of this series was carried out late in the season, when large changes would be unlikely.

The results from these series are not altogether conclusive. They are supplemented by two series carried out in 1932, the results of which are summarized in Tables XII and XIII.

Experiment S.A. 21 was carried out at a time when water contents were high, leaves not fully expanded and reserve water of the soil ample, and the changes appear to be minor ones.

The second series was carried out on August 24-25 during very hot weather. Very uniform figures were obtained just before sunrise on August 24. At 1 p.m. all four trees showed decreases of from 13 to 21% (average 17%), while at 7 p.m. increases (over the 1 p.m. results) of from 0 to 12% were

TABLE XII

Diurnal changes in water content. Birch (4-5 in. D.B.H. Height about 35 ft.). (Experiment No. S.A. 21)

Date	June 6		June 7			June 8
Time	2 p.m.	7:30 p.m.	4:30 a.m.	2 p.m.	7:30 p.m.	4:30 a.m.
Weather conditions, etc.	Fine, hot.	Fine.	Sunrise at 4:22 a.m. Cold, dull, windy.	Dull, slight shower	Fine.	Fine.
	76	84 (+11)	83 (−2)	84 (+3)	82 (−3)	84 (+3)

Bored breast-high to a depth of one inch. Averages from four trees.

TABLE XIII

Birch (4–5 in. D.B.H. Height about 35 ft.) (Experiment No. S.A. 22)

Date	August 24			August 25		
Time	5 a.m.	1 p.m.	7 p.m.	5 a.m.	1 p.m.	7 p.m.
Weather conditions, etc.	Sunrise 5:06 a.m. Fine.	Fine, very hot.	Fine.	Fine.	Slightly over-cast.	Fine.
	65	54 (−17)	58 (+7)	59 (+3)	50 (−15)	53 (+6)

Four trees.

recorded. At 5 a.m. next morning substantially similar figures (but well below the previous 5 a.m. figures) were obtained. At 1 p.m. decreases of from 9 to 21% (average 15%) were found, and at 7 p.m. somewhat less uniform figures showed an average increase of 6%.

In spite of the limitations of the method it is clear that when marked diurnal fluctuations are recorded the results are similar, *viz.*, a maximum water content at, or perhaps some time before, sunrise, a rapid decrease during the morning and an increase during the afternoon and night. This, as will be seen in a later paper, agrees with other observations, notably those on leaf water contents and changes in diameter of trunks.

There is one point that calls for note here. If rapid fluctuations in water content may occur, how reliable are the seasonal figures recorded above? The answer is that most of the results are from trees cut during the middle and later parts of the day. Borings have been taken almost without exception in the early afternoon and thus are comparable. The agreement between results from borings and those from the whole tree removes all doubt as to the possible effects of diurnal fluctuations on these figures.

Water Content of Diseased Trees

In trees of 8 in. D.B.H., red heart may extend over a diameter of two to three inches at the butt and the infection may reach the top logs. Where red heart is extensive a second condition, a brown rot, is often present, and is sometimes found even when red heart is absent. Samples from the Price Brothers limits have been examined by Miss Fritz (10), who finds that red heart is almost certainly due to *Torula ligniperda*, while a variety of organisms, including *Fomes igniarius*, *F. fomentarius*, *F. pinicola* and *Pholiota adiposa* may be associated with rot.

Infected parts are almost always much wetter than normal, as noted by Gibbs (11). The volume affected is usually relatively small and the flotability of an infected log will be but slightly less than normal. Infection through wounds is quite rapid and leads apparently to a higher water content in most cases (see discussion in section on girdling).

Snell (33, 34) has shown that for certain woods there are limits of moisture content at which fungi will grow. His "inhibition point of decay" corresponds to an air content of about 20% (based on fresh volume) of the woods. The gas content of wood in the living tree may be much below this value. In birch and in the sapwood of poplar it rises above and falls below this figure, while in the sapwood of the conifers considered it is normally less than 20%. In the heartwoods of poplar and of the conifers it is always (in healthy wood and except in the wet patches of balsam) considerably higher. In red heart of birch there is often much less than 20% of gas present and this, according to Snell, might be expected to inhibit further growth of fungi, but it is doubtful if this is the case.

Artificial Control of Water Content by Girdling

The effects of girdling are due primarily to two factors: the isolation of the root system from the source of organic food supply and (when girdling is deep) interference with the supply of water to the upper parts of the tree. Two major reasons for the girdling of hardwoods on pulp limits have been advanced and a certain amount of experimental work has been carried out with a view to commercial application by Churchill (5), Westveld (35), and Plice and Hedden (25). Where softwoods are desired it has been suggested that regeneration of conifers after selective cutting may be aided by the slow removal of the remaining hardwoods, and girdling is an obvious and practicable way of effecting this. If at the same time the water content of the girdled trees be markedly reduced, then the dead timber may be floated successfully and utilized for pulping.

In February 1930, birches were girdled on the Price Brothers limits, all wood being cut away to a depth of about one inch. Results are summarized in Table XIV.

TABLE XIV

WATER CONTENT OF GIRDLED AND NORMAL BIRCH

Girdled

Expt. no. Date, Remarks	Tree nos. and parts	Distribution of water across tree — Outside to centre					Disc
P.B. 11, June 14, 1930 (*i.e.*, girdled 4 mos.). Leaves nearly open.	1						
	Top	86	83	78	91	99	88
	Mid.	83	84	86	88	**115**	89
	Bt. A	91	93	97	**113**	**143**	94
	Bt. B	86	89	91	**115**	**132**	92
	2						
	Top	94	95	96	100	99	98
	Mid.	93	91	94	103	127	97
	Bt. A	94	87	94	**108**	**119**	94
	Bt. B	94	88	88	95	**130**	93
	3						
	Top	57	78	84	91	101	78
	Mid.	86	88	90	101	108	88
	Bt. A	86	88	98	**127**	**134**	94
	Bt. B	89	95	101	**119**	**135**	94
	Averages						
	Tops	79	85	86	94	100	88
	Mids.	87	88	90	97	—	91
	Bts. A	90	89	96	—	—	94
	Bts. B	90	91	93	—	—	93

Normal

Expt. no. Date, Remarks	Tree nos. and parts	Distribution of water across tree — Outside to centre					Disc
P.B. 7, Feb. 20, 1930. Cut at time and place of girdling.	Average of 3 trees						
	Tops	77	83	84	91	100	82
	Mids.	83	88	87	94	104	86
	Bts.	76	80	85	90	—	81
P.B. 13, June 25, 1930. From similar stand about three miles from girdled *plot.* Leaves fully open.	1						
	Top	100	91	92	92	100	97
	Mid.	99	91	90	91	**106**	99
	Bt.	93	90	94	97	**131**	96
	2						
	Top	101	96	93	97	102	102
	Mid.	93	92	96	97	112	96
	Bt.	89	91	90	**115**	**170**	106
	3						
	Top	93	90	90	91	103	95
	Mid.	89	87	91	**107**	**115**	93
	Bt.	84	80	84	95	**126**	87
	Averages						
	Tops	98	92	92	93	102	98
	Mids.	93	90	92	—	—	96
	Bts.	89	87	89	—	—	97

Bt. A = Butt sample above girdle; Bt. B = Butt sample below girdle. Figures in bold face are from blocks with red heart or with rot.

TABLE XIV—*Continued*

WATER CONTENT OF GIRDLED AND NORMAL BIRCH

Girdled

Expt. no. Date, Remarks	Tree nos. and parts	*Distribution of water across tree* Outside to centre					Disc
P.B. 23, June 6, 1931 (*i.e.*, girdled sixteen months). *Leaves* half open.	**1**						
	Top	47	65	74	88	96	62
	Mid.	63	76	78	97	109	73
	Bt. A	89	86	96	111	**141**	92
	Bt. B	99	85	94	**108**	**136**	95
	2						
	Top	54	73	79	83	91	68
	Mid.	82	80	83	91	104	82
	Bt. A	86	82	87	90	**142**	85
	Bt. B	92	86	89	88	**156**	90
	3						
	Top	51	72	85	101	92	66
	Mid.	63	85	93	107	**118**	80
	Bt. A	94	93	96	96	**106**	94
	Bt. B	97	*104*	*114*	*118*	**133**	105
	Averages						
	Tops	51	70	79	91	93	65
	Mids.	69	80	85	98	—	78
	Bts. A	90	87	93	99	—	90
	Bts. B	96	92	99	—	—	97

Normal

Expt. no. Date, Remarks	Tree nos. and parts	Distribution of water across tree Outside to centre					Disc
P.B. 23, June 5, 1931. From same stand as P.B. 13. Leaves nearly fully open.	**1**						
	Top	64	69	71	75	82	70
	Mid.	98	92	90	95	111	88
	Bt.	96	85	84	95	**112**	91
	2						
	Top	71	81	79	80	92	65
	Mid.	104	92	88	91	99	96
	Bt.	102	93	86	99	**138**	98
	3						
	Top	105	90	89	93	105	101
	Mid.	118	96	93	94	103	100
	Bt.	117	105	101	104	**134**	110
	Averages						
	Tops	80	80	80	83	93	79
	Mids.	107	93	90	93	104	95
	Bts.	105	94	90	99	—	100

Bt. A = Butt sample above girdle; Bt. B = Butt sample below girdle. Figures in bold face are from blocks with red heart or with rot.

TABLE XIV—*Concluded*

WATER CONTENT OF GIRDLED AND NORMAL BIRCH

Girdled								Normal							
Expt. no. Date, Remarks	Tree *nos.* and parts	Distribution of water across tree					Disc	Expt. no. Date, Remarks	Tree nos. and parts	Distribution of water across tree					Disc
		Outside to centre								Outside to centre					
P.B. 25, Aug. 15, 1931 (*i.e.*, girdled eighteen months). Leaves yellowing.	**1**							*P.B. 25, Aug. 17, 1931.* From same stand as P.B. 13.	**1**						
	Top	45	51	53	59	62	50		Top	59	53	53	56	63	56
	Mid.	43	48	53	60	74	49		Mid.	47	46	49	53	63	48
	Bt. A	59	54	52	56	79	56		Bt.	49	48	51	55	67	50
	Bt. B	62	61	55	56	78	60								
	2								**2**						
	Top	39	47	50	59	73	46		Top	63	58	59	62	70	61
	Mid.	42	64	81	82	78	56		Mid.	54	53	55	57	65	54
	Bt. A	45	40	51	61	**69**	47		Bt.	51	49	53	60	**110**	52
	Bt. B	66	51	62	64	**85**	60								
	3								**3**						
	Top	41	49	53	59	70	48		Top	56	53	55	57	65	55
	Mid.	43	61	84	95	106	62		Mid.	56	55	55	63	**75**	56
	Bt. A	52	75	105	109	**117**	75		Bt.	64	56	63	59	**101**	60
	Bt. B	70	96	108	**126**	**128**	91								
	Averages								*Averages*						
	Tops	42	49	52	59	68	48		Tops	59	55	56	59	66	57
	Mids.	43	58	73	79	86	56		Mids.	52	51	53	58	—	53
	Bts. A	52	56	70	75	—	59		Bts.	55	51	52	58	—	54
	Bts. B	68	69	75	—	—	70								

Bt. A = Butt sample above girdle; Bt. B = Butt sample below girdle. Figures in bold face are from blocks with red heart or with rot.

It will be seen that there is little difference in water content between normal and girdled trees. This is in agreement with the results of R. Hartig (15–17) for *Betula verrucosa* in Europe. The outer parts of trees above the girdle are definitely drier than those of the untreated trees, however, and this is certainly due to hindrance of the upward flow of water in these regions by the removal of the outer wood, but the reduction of water content is not sufficient materially to improve the flotability and even the slight improvement noted is counterbalanced by fungus infection.

While this may be a useful method of removing birch where softwoods are wanted, it is useless if utilization of the birch for pulpwood is desired. The results obtained are precisely as expected and one is forced to believe—experiments with dyes and on rapid fluctuations in water content seeming to confirm this—that the whole wood of the birch is available for water conduction.

Where there is definite heartwood and sapwood it has often been assumed that water conduction is confined to the sapwood. In poplar and balsam, however, where the heartwood, though drier on the average than the sapwood, contains a good deal of free water, it seems *a priori* possible that water can be carried through part at least of this region.

With a view to investigating the behavior of softwoods and particularly balsam, two trees of balsam and three of spruce were girdled on June 6, 1931, by cutting away all the sapwood in every case. These trees were felled and analyzed in September 1931. Results for balsam are summarized in Table XV.

TABLE XV

EFFECT OF GIRDLING ON WATER CONTENT OF BALSAM

Experiment no. Treatment, etc.	Remarks	Nos. of trees. Parts.	Water content				
			Sapwood			Heartwood	
			Outer	Middle	Inner	Wet patches	Dry patches
P.B. 23. Girdled June 6, 1931. Felled Sept. 15, 1931. All sapwood cut away.		1					
	Needles falling. Slight fungus infection (brown) near girdle.	Top	37	35	34	90	45
		Mid.	47	40	50	238	—
		Bt. A	36	37	33	262	72
		Bt. B	252	220	205	226	68
		2					
	Needles falling. Slight fungus infection (brown) near girdle.	Top	39	36	34	44*	36
		Mid.	45	55	47	160	47
		Bt. A	47	44	47	240	67
		Bt. B	276	241	212	266	55
Control P.B. 26. Freshly cut Sept. 15, 1931.	Water content substantially the same as that of girdled trees at time of girdling.	Average of three trees					
		Tops	223	215	189	180	—
		Mids.	251	229	208	246	42
		Bts.	248	211	190	287	42

*Bt. A = above girdle; Bt. B = below girdle. *No wet patches.*

The results from balsam are especially interesting. The sapwood above the girdles lost almost all free water but the heartwood still showed the characteristic wet regions noted earlier in this paper. These regions, then, cannot be considered as available for water supply.

The results from spruce were conflicting. In one tree almost complete drying of sapwood occurred but in the other two little change was noted. These were infected (subsequent to girdling) by a blue stain fungus. All three trees had substantially the same water content when girdled (borings were taken from different heights) and it is difficult to explain the results.

The experiment was repeated in 1932. Balsams behaved as in 1931 and three spruce trees showed various degrees of drying, but in no case did the sapwood remain as wet as in the two spruce trees examined in 1931.

The results for balsam resemble those obtained by R. Hartig (15–17) for oak (*Quercus pedunculata*) in Europe. He girdled two trees, removing a complete ring of sapwood in each case, and felled them one and five weeks after girdling. In each case the sapwood dried out very effectively while the heartwood was but slightly affected.

From the standpoint of flotability girdling is seen to be but partially effective. In the case of balsam, the top logs of which are notoriously poor floaters, girdling may be a remedy but it is questionable if it is a practical one. Birch is not materially improved. Spruce, with the exception of extreme top logs, floats well in any event and such drastic treatment as girdling would hardly be suggested for it.

Poplar, jack pine and larch have not been tested. The first has such a wide sapwood that removal would hardly be practicable, while jack pine floats fairly well and the quantity used, at least on the timber limits considered, hardly justifies investigation. The same may be said for larch.

Changes in the Water-Gas System of Trees and Logs during Seasoning and Flotation

In the previous pages the question of natural gas-water distribution in the living tree has been discussed. It remains to consider the changes which occur when the tree is cut and subjected to seasoning and flotation. It is obvious that the composition of the gas concerned as well as its volume will influence the rate of removal during flotation. MacDougal (19–21), and MacDougal, Overton and Smith (22) find that carbon dioxide is always present in amounts much greater than in the atmosphere (sometimes 60 times as great), while the amount of oxygen is much less, and the sum of oxygen and carbon dioxide less than in air.

It is not easy to determine variations in gas content since a decrease in volume of water may be followed by expansion of the gas present rather than by entrance of more gas. There are even workers who maintain that the conducting elements in the normal state contain water vapor rather than "gas" in the spaces not occupied by water. Scheit (29–32) believed this to be the case ("die wasserleitenden Organe entweder Wasser oder Wasserdampf, nicht aber Luft führen") and Priestley (personal communication) inclines to this view.

The very high tensions in wood vessels of the current year found by Priestley and confirmed by Scarth and Gibbs (unpublished work) show that the gas may be under extremely low pressure and it is quite possible that water vapor alone is sometimes present, a possibility that is rendered more likely by the quick refilling of vessels when transpiration is reduced.

Release of tensions, and of pressures when they exist, may lead to a change in the water-gas distribution in the tree, but the water contents of individual rings as determined from cut trees are so nearly equal, in top, middle and butt sections, as to suggest that the change, if any, is slight.

Fermentative and other changes, including those due to respiration of living cells in the sapwood, lead to changes in composition of gas in cut logs. This has been discussed by Boberg and Juhlin-Dannfelt (2, 3) and by Scarth and Gibbs (28). Scarth (27) testing wood from floated logs found very high concentrations of carbon dioxide.

(*a*) Field Seasoning of Birch

Attempts have been made to follow (*a*) the reduction in total water content and (*b*) the change in both lateral and longitudinal distribution of water, since distribution of the moisture is obviously almost as important as the quantity present.

Experiment P.B. 13. Started in June 1930

1. Three birch trees were cut, and the amounts and distributions of water were determined. Four-foot bolts were sawn from these trees, alternate logs were barked, and both barked and normal logs were piled together in the bush.

2. Three trees were felled, the trunks peeled, and the whole trees left lying, with the leafy tops on, in the bush.

3. In October the water contents of the trees and logs were investigated and fresh trees cut for comparison (Table XVI).

TABLE XVI

FIELD SEASONING OF BIRCH, 1930 (EXPERIMENT P.B. 13)

Date, etc.	Remarks	Position in tree	Distribution of water					Total water
			Outside to centre					
June A	Freshly cut. Three trees. Bolts used for C and D below.	Tops	98	92	92	93	102	98
		Middles	95	90	93	99	**111**	94
		Butts	88	87	89	**104**	**142**	93
		Average	97	90	91	(99)	(118)	95
June–Oct. B	Three trees cut same time as A. Peeled and left whole with tops on. Analyzed October.	Tops	31	33	35	34	33	34
		Middles	33	37	38	38	40	35
		Butts	35	40	44	50	**93**	40
		Average	33	37	39	41	(55)	36
Oct. E	Single tree felled in October. Analyzed at once.	Top	92	84	78	85	91	86
		Middle	90	82	81	77	88	84
		Butt	90	80	80	81	85	84
		Average	91	82	80	81	88	85
Nov. F	Three trees felled in November. Analyzed at once.	Tops	81	89	89	91	94	88
		Middles	83	85	83	88	103	84
		Butts	86	83	83	86	—	84
		Average	83	86	85	88	(98)	85
			Distance from end of log					Aver.
			0–1"	5–6"	10–11"	Middle		
June–Oct. C	4-ft. bolts from A, *with bark*. Piled June–Oct. Analyzed in October.	Tops	75	81	81	89		81
		Middles	59	74	80	89		76
		Butts	68	79	84	91		81
		Average	67	78	82	90		79
June–Oct. D	4-ft. bolts from A, without bark. Piled June–Oct. Analyzed in October.	Tops	59	36	37	37		42
		Middles	56	42	42	42		44
		Butts	56	44	45	45		46
		Average	57	41	41	41		44

Figures in bold face include those from blocks with red heart; averages bracketed include such figures or are incomplete.

In the four months which had passed the standing trees lost very little water (95% in June, 85% in October). The "sour-felled" trees, *i.e.*, those felled, peeled and left with tops on, however, had but 36% of moisture, the whole tree with the exception of the very centre of the butt being almost uniformly dry.

The peeled and unpeeled, piled logs, which had been piled on skids to keep them free of the ground, had lost very different amounts of water, the unpeeled logs having almost as much water as the standing trees (79 against 85), while the peeled logs had lost more than half their water (from 95 to 44%).

The first snowfalls of the autumn occurred while the October analyses were being made and very quick penetration of water (from early, wet snow) was taking place through cracks which had developed in the peeled logs and in the peeled sour-felled trees.

The summer of 1930 had been a relatively wet one, so the experiment was repeated in 1931 which proved to be a much drier year. The results are summarized in Table XVII.

TABLE XVII

FIELD SEASONING OF BIRCH, 1931 (EXPERIMENT P.B. 23)

Date, etc.	Remarks	Position in tree	Distribution of water: Outside to centre					Total water
June	Three trees. Freshly cut.	Tops	80	80	80	83	93	79
		Middles	106	93	90	93	104	95
A		Butts	105	94	90	99	128	100
		Average	97	89	87	92	(108)	91
June-Sept.	Three trees cut at same time as	Top*	52	42	43	43	46	42
	A. Peeled and left whole with	Middle*	29	32	37	36	36	36
B	tops on. Analyzed in Sept.	Butt*	33	37	41	63	89	37
		Average	38	37	40	47	57	38
July-Sept.	Two trees felled in July. Both	Top 1	—	—	—	—	—	48
	left whole with tops on. No. 1	Top 2	—	—	—	—	—	59
E	peeled. No. 2 with bark on.	Mid. 1	36	35	38	37	38	37
	Analyzed in Sept.	Mid. 2	47	44	47	50	56	45
		Butt 1	—	—	—	—	—	45
		Butt 2	—	—	—	—	—	45
		Average 1	—	—	—	—	—	43
		Average 2	—	—	—	—	—	50
Sept.	Three trees felled in September.	Tops	54	55	57	57	67	56
		Middles	57	59	60	63	72	60
F		Butts	69	70	72	77	82	71
		Average	60	61	63	65	74	62
			Distance from end of log: 0-1″	5-6″	10-11″	Middle		Aver.
June-Sept.	4-ft. bolts from A (above) with	Tops†	61	73	79	77		73
	bark, left piled in bush.	Middles†	66	68	74	82		73
C	Analyzed in September.	Butts†	68	77	81	84		76
		Average	65	73	78	81		74
June-Sept.	As C but without bark.	Tops†	47	37	37	39		40
		Middles†	46	40	41	40		42
D		Butts†	47	46	47	48		47
		Average	47	41	42	42		43

**Top from tree 1, middle from tree 2 and butt log from tree 3 only, but total water calculated from all logs.*

†Logs from trees 2 and 3 only.

During 1931 the standing trees lost very much more water than in 1930, otherwise results were much as in 1930. In both experiments the sour-felled peeled trees had shown the optimum seasoning and since in June it is remarkably easy to peel birch with a "spud" the method has certain advantages. It seems fairly certain that it is evaporation from the leaves that leads to the better seasoning of sour-felled trees.

Flotation tests which are described below were carried out in an effort to determine the fate of such seasoned logs.

(b) Flotation

1. *Top-drying*

Flotation under laboratory conditions may be a very different thing from flotation under commercial conditions. It must be remembered, however, that the passage of logs from the bush to the mill (the "drive") is not always a continuous process. Logs in the Shipshaw river, for example, may be boomed for months in Lac Onatchiway before being cut into shorter lengths ("slashing") for driving to the mill at Kenogami. Here there might well be a great measure of top drying similar to that observed in early laboratory experiments (Scarth and Gibbs (28)). Analysis of logs from the boomed timber did not confirm this but indicated rather that there must be considerable turning of logs during flotation. This was estimated on a small scale by booming a set of marked logs during the summer of 1931. These were selected in part from timber which had already been in the early part of the drive and in part from freshly cut material. The logs were first boomed in June and examined in July, August and September. Of the thirty logs floated, no fewer than nine (or 30%) had turned to some extent before September and analysis of several of the logs, including turned and unturned samples, indicated very little top drying.

2. *Experiments under Laboratory Conditions*

Experiments designed to test the relative importance of end and side penetration were described in an earlier paper (28). End penetration is of great importance and it was shown that painting would very considerably reduce penetration, although, when solution and exodiffusion of enclosed air are the limiting factors, end penetration is of less significance (27).

The rapid seasoning of peeled logs very naturally led to an investigation of their behavior during flotation, while the question of end penetration suggested a consideration of log length.

(a) Barking and painting (birch and balsam). Each freshly cut log, as received, was examined for water content and divided to form a pair, as follows:—

Birch Log 2A—Floated with bark.
2B—Floated after peeling.
5A—Peeled, ends painted, then floated.
5B—Peeled, sides painted, then floated.
8A—Peeled (discarded by accident).
8B—Peeled, painted all over, then floated.

The series was started in November 1930 and increase in weight was recorded at irregular intervals until early October 1931. At this time only one log (8B) remained afloat; 5B started to sink in January, while the other logs were all down by the end of April.

Balsam Log 2A—Barked, ends painted, then floated.
2B—Barked, sides painted, then floated.
5A—Floated with bark on.
5B—Barked, painted all over, then floated.
8A—Barked, then floated.
8B—Barked, then floated.

The results from both series are summarized in Fig. 11, in which increase in weight, expressed as a percentage of the weight when floated, is plotted against time. In each series the lowest curves (8B and 5A in 11A; 5B and 2A in 11B) are those for logs with painted ends. These show approximately a straight line relation between increased weight and time of flotation. The curves for all other logs indicate a more rapid intake of water in the earlier stages of the experiment, followed by a decreasing rate of penetration. In 5A (series B), indeed, there was actually a slight decrease in weight during the summer. This was probably due to evolution of gas by fermentation (see 2 and 3).

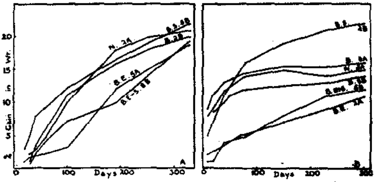

FIG. 11. *Increase in weight of floated logs. Birch series A, balsam B. Letters on curves:* B = *barked.* N = *normal (with bark).* E = *ends painted.* S = *sides painted.* E + *S* = *ends + sides painted.*

Evidently removal of bark from unseasoned logs has very little effect on the rate of penetration of water (2A and 2B of the birch series). Later experiments with seasoned logs yielded somewhat different results (see below). The prevention of top drying which follows painting of the sides of logs is evident in the case of Log 2B in the balsam series. It is less obvious in Log 5B of the birch series, but even there it is clear that the water content is still rising steadily in October, while the rate of increase of weight of the other logs is falling off.

(*b*) *Barking, painting and log-length (birch and balsam).* One-, two- and three-foot sections were cut from eight-foot logs. The treatments and results are given in tabular form in Tables XVIII and XIX.

The conclusions to be drawn from the figures are not altogether clear. This is due in part to the limitations imposed by the nature of the experiments. Certain facts, however, are clear. The longer logs season more slowly than the shorter ones but the difference after 100 days under these conditions, in a fairly cool, well ventilated room, is slight. Logs from which bark has been removed show no difference due to log length at the end of this time. Barked logs season more rapidly than normal logs. When floated,

there is a certain amount of side penetration which is reduced by painting. In the present series there was prolonged seasoning and almost no top drying during flotation, so that the effects of side painting in preventing side penetration are clearly brought out. In the previous series, with unseasoned logs, top drying was extensive. The very marked effect of end painting is evident here, more especially in the balsam series.

TABLE XVIII

Seasoning and flotation of birch, 1931. Effect of log length. Experiment started March 8

Log nos.	Days from beginning of experiment to date	Treatment, etc.	Weight as percentage of original weight	
			1-ft. log	3-ft. log
A1* and A2	60 (May 7)	*Seasoning with bark on*	76	84
	110 (June 26)	*Seasoning with bark on*	72	80
	134 (July 20)	*Seasoning with bark on.* Then *floated July 20*	70	78
	210 (Oct. 9)	**Flotation about ½ in.	106	100
B1 and B2	60 (May 7)	*Seasoning without bark*	65	68
	110 (June 26)	*Seasoning without bark*	64	66
	134 (July 20)	*Seasoning without bark.* Then *floated July 20*	64	65
	210 (Oct. 9)	Both sunk before Oct. 9	113	116
C1 and C2	60 (May 7)	*Floated from March 7 with bark on.* C1 sunk, C2 with one end down.	114	107
	110 (June 26)	Both logs had sunk	119	115
	134 (July 20)	Both logs had sunk	120	117
	210 (Oct. 9)	Both logs had sunk	121	121
D1 and D2	60 (May 7)	*Seasoning without bark*	67	68
	110 (June 26)	*Seasoning without bark*	66	66
	134 (July 20)	*Seasoning without bark.* Then *sides painted and floated on July 20*	66	66
	210 (Oct. 9)	D1 had ¼ in. flotation, D2 had one end down	117	117
E1 and E2	60 (May 7)	*Seasoning with bark on*	74	81
	110 (June 26)	*Seasoning with bark on*	70	76
	134 (July 20)	*Seasoning with bark on.* Then *ends painted and floated on July 20.*	69	75
	210 (Oct. 9)	E1 flotation 1 in., E2 flotation 1¼ in.	84	93

* *The first log of each pair is the 1-ft. log, the second the 3-ft. log.*

** *"Flotation" indicates amount of log above water.*

TABLE XIX

SEASONING AND FLOTATION OF BALSAM, 1931. EFFECT OF LOG LENGTH

Log nos.	Days from beginning of experiment to date	Treatment, etc.	Weight as percentage of original weight		
			1-ft.	2-ft.	3-ft.
A1*,	50 (May 7)	*Seasoned with bark on*	59	63	71
A2	104 (June 29)	*Seasoned with bark on. Floated July 4.*	50	53	58
and A3	206 (Oct. 9)	Flotation: A1 = 1 in., A2 = $1\frac{1}{3}$ in., A3 = $1\frac{3}{4}$ in.	91	81	78
B1,	50 (May 7)	*Seasoned without bark*	48	49	50
B2	104 (June 29)	*Seasoned without bark. Floated July 4.*	46	46	47
and B3	206 (Oct. 9)	Flotation: B1 = 1 in., B2 = $1\frac{1}{4}$ in., B3 = $\frac{3}{4}$ in.	97	88	93
C1,	50 (May 7)	*Floated March 17 with bark*	123	118	117
C2	104 (June 29)	Floating	124	120	120
and C3	206 (Oct. 9)	C1, one end down Flotation: C2 = $\frac{1}{2}$ in., C3 = $\frac{1}{2}$ in.	126	122	122
D1,	50 (May 7)	*Seasoned with bark*	62	70	75
D2 and	104 (June 29)	*Seasoned with bark. Barked and floated on July 4.*	50	58	64
D3	206 (Oct. 9)	Flotation: D1 = $\frac{3}{4}$ in., D2 = $1\frac{1}{3}$ in., D3 = $1\frac{3}{4}$ in.	88	72	71
E1,	50 (May 7)	*Seasoned without bark*	48	54	55
E2 and	104 (June 29)	*Seasoned without bark. Sides painted then floated on July 4.*	46	48	48
E3	206 (Oct. 9)	Flotation: E1 = $1\frac{1}{3}$ in., E2 = $2\frac{1}{4}$ in., E3 = $1\frac{3}{4}$ in.	89	74	75
F1,	50 (May 7)	*Seasoned with bark*	63	74	75
F2 and	104 (June 29)	*Seasoned with bark. Ends painted then floated on July 4.*	53	61	60
F3	206 (Oct. 9)	Flotation: F1 = 3 in., F2 = $2\frac{3}{4}$ in., F3 = $2\frac{1}{4}$ in.	60	66	63
G1,	50 (May 7)	*Seasoned with bark*	53	57	65
G2 and	104 (June 29)	*Seasoned with bark. Barked, sides painted then floated on July 4.*	46	49	56
G3	206 (Oct. 9)	Flotation: G1 = $1\frac{1}{4}$ in., G2 = $1\frac{1}{3}$ in., G3 = $2\frac{1}{4}$ in.	78	69	69

* *In each group the first log is the 1 ft., the second the 2 ft. and the third the 3 ft. log.*

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STUDIES OF WOOD

III. ON THE PHYSIOLOGY OF THE TREE, WITH SPECIAL REFERENCE TO THE ASCENT OF SAP AND THE MOVEMENT OF WATER BEFORE AND AFTER DEATH[1]

By R. Darnley Gibbs[2]

Abstract

The various theories of the ascent of sap are discussed and it is concluded that the tension hypothesis best meets the observed facts. Seasonal and diurnal variations in water and gas content and the changes in tension that are demonstrable are all in accordance with the demands of the tension hypothesis. The two greatest problems are probably the maintenance in conducting channels of continuous water columns under high tensions without entry of air, and the refilling with water of such channels as do become gas-filled during the summer (for the insulation of gas and water channels one from the other is apparently not perfectly maintained). These problems are not entirely settled, though the hypotheses of Münch and/or Lund may account for the refilling of vessels in deciduous trees during the non-leafy season.

The observed differences in the behavior of maple, birch and poplar during the period of sap flow have received attention. These peculiarities cannot be accounted for by differences in the root systems. The earlier cessation of bleeding and the development of tensions in poplar are correlated with a higher rate of evaporation from the twigs of the former, and this evaporation probably is largely through lenticels.

The problem of sinkage, in the light of this work, is briefly reviewed.

In a previous paper (55), the results of work on water content of trees and on the changes in water content that follow cutting have been presented. It remains to discuss these results in the light of present day knowledge of the physiology of the tree, and the discussion may well open with a consideration of theories bearing on the ascent of sap.

a. The Living Cell ("Vital") Theory

It is customary to consider that the works of Malpighi (see Sachs (141) and Grew (58, 59)) in the 17th century mark the beginnings of the science of plant physiology. It was natural in such "vitalistic" times that Grew should postulate a pumping action by living cells—an action that is still considered by a minority of workers in this field to explain the ascent of sap. Grew undoubtedly considered that sap flow, in part at least, took place in the "bark" (phloem and cortex) but he recognized that the wood also contains water at certain times: "For in the beginning of spring, it (the sap) riseth, neither betwixt the Wood and Bark, nor in the Bark; but only in the Wood."

His theory as to the ascent of sap, however, would seem to apply to the bark, and his "vessel" is not to be confused with a "wood vessel" as understood today. It is significant that he dismissed capillarity as an adequate force and regarded the parenchyma cells surrounding the "vessels" as pumping water into them.

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Contribution from the Department of Botany, McGill University, with financial assistance from the National Research Council of Canada. From a thesis approved for the degree of Doctor of Philosophy in the University of London, England.
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Westermaier (197, 198) decided that the conducting system of woody plants consists either of living wood parenchyma or of a combination of living and dead cells. Godlewski (57) differed on some points from Westermaier: "*Nach mir . . . Hadromzellen im Holze nur als Stempel der Saugdruckpumpen . . . die Gefässe . . . die Röhren . . . nach Westermaier die Hadromzellen die Strombahn des Wassers selbst, und die Gefässen . . . als Reservoire.*" Godlewski's theory was adversely criticized by Zimmermann (207). Westermaier evidently regarded the wood parenchyma ("hadrome") cells as the conducting tissue, while Godlewski thought of the wood vessels as carrying out this function and the parenchyma cells as supplying them with water—*an entirely different thing when considered from the standpoint of the velocity* of conduction. The rate at which water actually moves is, of course, very important when considering the manner of conduction. Huber (77) slightly warms a small region of the stem and then detects the propagation of the wave of increased temperature by means of tiny thermocouples. This probably gives one of the closest measurements of the actual rate and with it he has obtained a value as high as 75 cm. per minute—a velocity that is many times the possible maximum of diffusion from cell to cell.

A number of investigators, including Bierberg (9), who estimated that protoplasmic streaming accelerated movement of water three to four times, and Hugo de Vries (195), have postulated protoplasmic streaming as a factor in hastening transfer of water and solutes, but even with the most rapid streaming observed the rate of transfer could not be adequate.

Many others have concluded that living cells play some part in sap ascent. The views of Janse (83, 84), for example, are very much like those of Westermaier and Godlewski referred to above. In his early work, Ursprung (181), later a staunch supporter of the tension hypothesis, decided after a study of about twenty woody plants, that with the possible exception of *Sorbus aucuparia*, living cells played a part in all cases. C. H. Schultz (151) believed vital processes entered into the phenomenon, as did Boehm (14), Nordhausen (121) and Reinders (133, 134). Eames and MacDaniels (41, p. 68) say that "Histological evidence strongly suggests that the presence of living cells is necessary to the upward conduction of water by tracheids and vessels. Every water-conducting cell is in contact in some part of its wall surface with one or more living cells, and abundant pits are present in this contact area."

Ranged against these are many opponents of vitalism. Vesque (194) and Strasburger (168, 169), in his classic experiment with poisoned trees, presented arguments against the "vital" theory, while E. F. Smith (157) and Overton (122) observed flow of water through dead stems. Curtis (25, 26, 27) chilled petioles to check translocation and noted that movement of water was not appreciably decreased, an observation which it would be hard to reconcile with transfer by living cells. Zijlstra (205), nineteen years earlier, noted a similar lack of effect in *Helianthus* (Curtis used *Phaseolus vulgaris*), while Polunin (125) found that roots of *Betula odorata* conduct water at 0° C. Smith, Dustman and Shull (158) also refuse to believe that living cells have a role in sap ascent.

Although water will continue to pass through a dead stem for some time, the flow frequently decreases and becomes inadequate. The vitalists claim that this is due to the absence of living cells, the non-vitalists declare that the cause is occlusion of the dead water-conducting cells by material derived from the cells killed by the experiment. Not only are the living cells of no use in sap ascent, but in their death they prevent it! Dixon (31, 32) and his co-worker, Joly (36, 37, 38, 39), have given much thought to the question. Dixon concludes that the changes occurring above the killed section are due to the toxic action of the sap from the dead cells rather than to the cutting off of the water supply.

b. The Imbibition Theory

The fact that the cell walls of wood readily absorb a considerable amount of water led early investigators to believe that movement of water takes place in the walls. Unger (179, 180), and Wiesner (200) were among the supporters of this "imbibition theory" and Sachs (141) in his early work also believed in it, but he recanted later. Elfving (44), who investigated the relative movement of water in the radial, tangential and longitudinal directions, argued against imbibition as did Boehm (14).

Work on the changing water-content of floating logs, during which top-drying to well below the fibre saturation point has been noted, and the persistence of free water in the wet patches of balsam heartwood (Gibbs (55)), prove that the movement of water under such conditions is far from easy. Although imbibition may have relatively little to do with the ascent of sap it is by no means unimportant in the general economy of the plant, as Shull (155) and Woodhouse (202) point out.

c. The Capillarity Theory

Although the height to which water will ascend by capillarity in tubes of the diameter of wood vessels is insignificant when compared with the heights to which it rises in trees, many of the early investigators were misled by conditions in the tree and considered that capillarity could explain the ascent of sap. The presence of numerous air bubbles alternating with short columns of water (the so-called "Jamin chain") was supposed to decrease the effective hydrostatic head and make possible the easy ascent of water to heights exceeding that of a continuous column of water balanced by atmospheric pressure. Jamin (82) is usually credited with (or blamed for) the development of the theory. He stated, in a paper dated 1860, that "les forces capillaires suffisent pour expliquer le mouvement de la sève". Schwendener (153, 154) considered the process from the practical and from the mathematical viewpoints and supported Jamin. He took borings from pine, beech, etc. and found air and water in the vessels. Zimmermann (206), who seems to have criticized all the theories then current, failed to agree with Jamin. In the same year Vesque (193) published the results of observations on *Tradescantia zebrina*, and claimed that air obstructs rather than aids the movement of water in the vessels, while Pappenheim (123) noted that air bubbles in tubes may not move and attempted without success to demonstrate the

movement of water past the bubbles, though Strasburger (168) claimed (as did Copeland (23) later) that water does pass bubbles in the tracheae. The last remarks that if the bubbles moved the whole problem would have been solved by Malpighi! All recent work would seem to condemn the Jamin chain hypothesis.

d. The Distillation Theory

It has long been known that "tensions" may exist in the vessels of rapidly transpiring trees. If water be supplied from below and removed from above—with air or water vapor under low pressure between—then a relatively rapid distillation may occur. Scheit (145, 146, 147, 148) seems first to have advanced this "distillation theory". Priestley (personal communication) considers that it may play a part, and it is true that some vessels of rapidly transpiring trees may contain air under very low pressure—or perhaps nothing but water vapor. Against the theory, however, is the fact that substances in solution rapidly ascend to the tops of trees more than 32 feet high.

e. The Liquid-tension Theory

The idea that liquids might be drawn up to considerable heights by suction from above met with much opposition on the ground that the maximum height would be (for water) but little over 30 feet. In 1850, however, Berthelot (8), experimenting with a closed tube full of liquid, found that the liquid on cooling did not part from the tube and leave a "Torricellian vacuum" but "dilated" and continued to fill the tube. It was soon shown that the "tensiie strength" of water actually amounted to several hundred atmospheres but the facts were not at once applied to biological theory, though Spencer (160), in a curious paper which postulated wind-produced oscillation of the tree as effective in causing sap ascent, said that: "A state of capillary tension must result . . . resisted below by liquid cohesion". In 1893, Boehm (15) claimed to have observed the lifting of mercury to over 90 cm. by a transpiring *Thuja* twig. His experiments were followed by those of Dixon and Joly (36, 37, 38, 39) who boldly proclaimed that *ascent of sap is brought about by evaporation from the leaves acting through liquid columns under tension and that the presence of dissolved air does not destroy the continuity of the columns*, and Askenasy (1, 2) who demonstrated a lift of more than 82 cm. of mercury by evaporation from a plaster-filled tube and applied the idea to ascent of sap.

The tension theory presents a number of grave difficulties. First of all is the need for the demonstration of *continuous water columns from leaf to root*. These, if present, may be very easily ruptured and this is one of the major arguments against the cohesion theory. Ursprung (183), in 1913, claimed that sap had very little tensile strength, but Dixon (33) found it able to withstand tensions of more than 200 atmospheres, nor did dissolved air decrease this very significantly. Measurement of the tensile strength of the liquid in the fern annulus and in the elators of a liverwort (*Lophozia*) were made by Renner (135), whose figures agree very closely with those of Dixon, while Ursprung (187) in his later work found tensions up to 300 atmospheres in fern annuli.

Water can be drawn up by evaporation from a porous clay surface, or even from a living twig, using a long, unobstructed tube or one filled with plaster of Paris, and lifts of more than 76 cm. (using mercury) may be obtained. The experiments of Askenasy have been mentioned above, but see also Livingston (96), Ursprung (182, 183, 184, 186) and Nordhausen (119, 120, 121). Copeland (23) described an artificial tree, complete with branches, of plaster-filled tubes, and his comments on the operation of this deserve quotation: "The positive result of our experiment is that the water column being continuous, but air being present, a suction of less than one atmosphere can still operate as a suction more than 12 metres down." (p. 166). And "This artificial tree had so much in its behaviour in common with real trees that when all was done I was likewise unable to explain it." Dixon, however, showed that the rate of transfer in Copeland's tree was hopelessly inadequate.

But to return to the subject of continuous water-columns. Bode (13) found unbroken columns in *Impatiens*, *Zebrina pendula*, *Cucurbita*, *Elatostemma*, etc. By direct microscopic examination he observed the movement of sap in the vessels and saw no air bubbles at all in uninjured water-conducting cells. He realized that some vessels may contain gas, but considered the actively conducting cells to be completely filled with water. Holle (72) some years earlier had found continuous water columns in other plants. The work of MacDougal (102, 103), MacDougal and Shreve (105) and MacDougal, Overton and Smith (104) also points to the existence of continuous water columns (see below).

Observations on the water content of birch at different seasons, and on the amount of water in individual year-rings of birch, larch, balsam and spruce indicate that the columns of water are continuous in these cases also. The similar water content of birch, at breast height and at 20 feet from the ground (Table I), certainly points to this conclusion.

TABLE I

BIRCH AT STE. ANNE'S (D.B.H. 4–5", HEIGHT ABOUT 30 FEET). BORED AT BREAST-HEIGHT (BUTT) AND AT ABOUT 20 FEET FROM THE GROUND (TOP) TO A DEPTH OF ONE INCH

Date, time	Position in tree	Water content of individual borings									Averages
		Tree no.									
		1	2	3	4	5	7	8	A	B	
May 3 1–2 p.m.	Top Butt	88 83	102 104	101 98							97 95
May 14 2–3 p.m.	Top Butt				87 88	82 80	79 76	99 94			86 84
May 19 2–3 p.m.	Top Butt				78 82	72 78	80 68	81 80			78 77
Aug. 25 1–2 p.m.	Top Butt	54 53	45 51	51 52							50 52
Oct. 1 2–3 p.m.	Top Butt								58 48	53 58	56 53

It is realized, of course, that practically uniform water content might result from a purely random distribution of very short columns, but it would appear unlikely that this would be maintained so constantly. The surprisingly uniform results obtained from the same rings at different heights in the tree (Fig. 1) are also of interest in this connection.

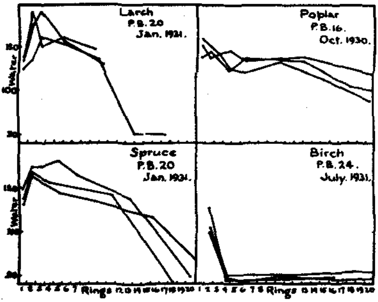

FIG. 1. *Water in annual rings of several trees. Each set of three curves represents results from top, middle and butt samples. The numbering of rings is from the outside.*

Direct observation with the naked eye does not suggest the regularity of distribution of gas and water within the single ring observed by MacDougal (102, 103), but it is not difficult to reconcile this with the presence of continuous water columns (see also Baker and James (7)).

Another difficult problem associated with the tension hypothesis is the actual demonstration of very high tensions. Unfortunately this issue has been much confused, since few of the actual workers seem to have realized that the complex organization of the stem is such that separate (and largely independent) systems may exist side by side at one and the same time. Manometer measurements, which have been largely quoted, are evidently of little value. Since the time of Boehm (14, 15) many have attempted to demonstrate tensions and some of this work has already received notice above. Ursprung (188) claims to have demonstrated the formation by tension of continuous mercury columns of more than 76 cm. in branches of *Clematis vitalba*, using X-ray photography to record his results, while Thut (173, 174) obtained lifts, using living twigs attached to glass tubes after the manner of Boehm, of as much as 226.6 cm. of mercury (ca. 3 atm.). Nordhausen (120) attempted to measure the suction by comparing the rate of flow induced by the living twig with that produced by a pump of known efficiency and attached to the same stem as the twig, obtaining values up to 8 atm. by this means, while Renner (136) obtained a maximum value of 9 atm. for *Forsythia*.

When tensions are developed in the cells, a corresponding flexure of the cell wall results, and this may be detected and measured (13). Measurements of diameter changes of trees have shown that increase in diameter during growth is not a steady thing but that rhythmic diurnal changes may occur. MacDougal and his associates, Overton, Shreve and Smith (104, 105), have used the "dendrograph" for such work and attribute the smaller, rhythmic fluctuations in the form of the curves obtained to tension changes. Kraus (90), as far back as 1881, noticed that stems shrink during the day and

swell again at night, while Friedrich (quoted by Büsgen (18)) correlated changes in stem diameter with evaporation and noted accompanying small diurnal changes in water content. Mallock (108) used a very ingenious optical method to detect such small variations in growth. According to Haasis (61), a progressive contraction may occur during the dry season in the case of Monterey pine (*P. radiata*) and redwood (*Sequoia sempervirens*) with small increases at night (but see also the discussion below on seasonal variations in conifers). The diurnal variations observed agree in general with the daily fluctuations in water content of the tree.

The development of high tensions introduces a new, and perhaps the greatest, difficulty associated with the tension hypothesis. This is the question of the *permeability of the wood to gas. How are the gas and water systems isolated? Why does not gas pass into the conducting vessels when the pull is developed?* Intimately connected with these questions is that of the conductivity of wood for water. Most of the measurements of conductivity have been made with relatively large pieces of stem or even with isolated chips of wood—but *the conductivity of the cut stem or the permeability to gas of a block of wood tells little of the state of affairs in the living plant.*

Groom (60) and Farmer (47) have compared the rates of flow in conifers and deciduous trees, while detailed investigations of different species have been made more recently by Holmes (73, 74) for hazel and ash, by M. and F. Rivett (139) for *Rhododendron ponticum* and *Ilex aquifolium*, by Inamdar and Shrivastava (78, 79) for some tropical species, by Furr (52) and by Malhotra (106, 107). It would appear from this work that the minimum pull required to draw water to the top of a tree is equivalent to a hydrostatic head of about twice the height of the tree, but if the conductivity of the actual conducting portion be less than that of the stem as a whole the figure will be still higher. This means that in a tall tree tensions of at least 20 atm. must be developed. Why does not gas pass into the conducting cells under these conditions?

Steinbrinck (167) claims to have forced air through walls (?) of wood with a pressure of about one atmosphere, as did Jost (87). Claussen (20) found that wood is increasingly permeable as its water content rises, and his results would seem to be confirmed by those of Porsch (127). In 1910, however, Tiemann (176) claimed that ". . . in the fresh green wood . . . the cells . . . (except the resin ducts and the vessels) are completely closed by the continuous primary wall . . . and that gases cannot be forced through this enclosing membrane, even at extreme pressures." He thought that the increased permeability of wood seasoned to below the fibre saturation point is due to the formation of minute slits in the walls, a view adversely criticized by Bailey (3, 4, 5, 6), who showed that the pit membranes (part of the "continuous primary wall" of Tiemann) are not entire but are perforated. They are greatly changed by drying but are not ruptured by a pressure of 250 lb. to the square inch. According to Bailey ". . . the valve-like action of the tori undoubtedly assists in retarding the entrance of air into the water-conducting passages". If the tori do not act, however, the surface tension

of the sap in the pit membranes can be overcome by pressures of less than 3 atm. Incidentally, Miss Wright investigated the pit-closing membrane of the lower gymnosperms (203) and found that in *Ginkgo*, which becomes a large tree, the torus may be only half the width of the pore, so that it could not exert a valve action.

The actual dimensions of the pores of the pit membranes have been established by Bailey (3, 4, 5, 6,), Stamm (164, 165, 166) and others. They are of such a size that pressure of perhaps 3 atmospheres should cause passage of air (see below). During the course of penetration studies, Sutherland (172) came to the conclusion that ". . . little resistance to flow is encountered in pit membranes of sapwood, and that the tracheids themselves provide the greater part of the resistance." It is difficult to believe that this is so. Sutherland claims to have found no evidence of the tori acting as valves.

In a recent address (131) Priestley remarks: "In actual experiments we have failed to displace the liquid contents of closed hardwood vessels by air, using pressures of 15 atmospheres. . . . Pores of pits do not open on to intercellular spaces . . . the liquid contents of the tracheal elements may be under tension without the slightest likelihood of air being drawn in from the intercellular system of the wood . . ." Priestley is dealing with the living tree and not with isolated blocks of wood: his results are therefore much more likely to give a true picture of conditions in the tree and if his figure of 15 atm. be correct, tensions could lift water to at least 220 ft. (reckoning the necessary hydrostatic head at 2 $\times$ height of tree) without drawing air into the conducting cells.

Let us return again to a consideration of the mechanism that produces the pull on the water columns of the stem. The general opinion would seem to be that the water columns of the vessels are continuous with the living cells of the leaf and that these cells suck water from the vessels. Ursprung and Blum have contributed a great deal to this subject and their conception of suction force ("saugkraft") as the effective force has made an understanding of the subject much easier. Some of Ursprung's work has been mentioned above and further details are to be found in other papers (185, 189) by him, by Ursprung and Blum (190, 191, 192) and by Huber (75, 76). Dixon (34) and Miss Ernest (45) criticize the methods for the measurements of "saugkraft' and consider that many of the values given in the literature are incorrect, owing to changes in tensions, etc. during the course of the measurements. Very high osmotic pressures, however, have been recorded in a number of cases. Among the most interesting of these are those due to Fitting (49) who studied many Sahara desert plants, no less than 21% of which had osmotic pressures of 100 atm. or more, and Harris, Gortner and Lawrence (64), working in the Arizona desert, who measured the depression of the freezing point of the sap of different classes of plants. The last obtained average values for Δ of 2.34 (equivalent to an osmotic pressure of 28 atm. for trees and shrubs, 1.733 for dwarf and half shrubs, 1.357 for perennial herbs and 1.227 for winter annuals.

Although there is abundant proof of very marked tensions in plants and of the importance of transpiration in developing these tensions, active secretion of water may occur in the absence of transpiration. The existence of an apparent transpiration stream in submerged aquatics suggests a secretion of water by the (morphologically) upper parts of the plant. Thut (175), however, concludes that ". . . the flow of water through the submerged plants studied thus seems to be due primarily to 'root-pressure'." Active secretion is not confined to aquatic plants, being well known in land plants as "guttation".

It is convenient to turn again to the question of water content of trees as the points that remain to be discussed can be considered in this connection.

Some of the earliest results are those due to Matteucci (110), who cut poplar during a rigorous winter and found an average of 60% of water in the root, 56% at four metres and 51% at eight metres. His figures are too few to be of much use. In 1876, Geleznow (53) published the results of numerous observations in Russia on *Betula alba*, *Populus tremula*, *Acer platanoides* and *Pinus sylvestris*, trees closely related to those studied and described in the second paper of this series (55). His results are somewhat irregular but he noted a summer drop in the case of the hardwoods, while the one conifer given (*Pinus sylvestris*) showed very small changes in water content. In their extensive studies on sap flow in the maple, Jones, Edson and Morse (86) give the following figures* for water content of maples: December, 31.5, March 15, 36.5 and April 28, 47%. This rise was followed by a rapid decrease after the buds opened. Büsgen (17) and his students noted diurnal fluctuations in water content. Their figures for larch are particularly interesting, as they suggest that larch behaves as do other deciduous trees rather than as a conifer.

What are the causes of the seasonal changes, and why do trees, growing apparently under the same conditions, differ one from another in their water relations?

Broadly speaking, the climatic factor is one of the most important of all. In temperate regions with a summer growth period and a winter rest, or in parts of the tropics where a dry season takes the place of the winter, there is a season of rapid transpiration and, usually, a water deficit, followed by a period during which either little change in water content may be expected or the deficit is made up. It is generally conceded that the deciduous habit is a response to this and that the evergreen habit involves xerophytism in some form. A re-examination of observations on water content from this point of view is of interest.

Birch and poplar agree in showing a high water content at leaf opening followed by a relatively rapid, but by no means uniform, decrease during the summer months. During 1931, for example, the water contents of birch and poplar dropped from 92% (early June) to 54% (August), and from 124%

The figures were really due to Spaulding (159), to whom thanks are due for the loan of manuscript records.

(late April) to 68% (September) respectively. There is no doubt that this loss is due to evaporation from the leaves at a rate in excess of water absorption. In 1930, on the other hand, a much less marked fall occurred. What are the factors responsible for this difference? The answer is by no means certain, but among the major factors are precipitation and humidity.

The period June-July of 1931, during which the water content of birch fell from 92 to 54%, was not an unusually dry spell, nor was the humidity low. August 1931, however, was much drier than the same month of 1930, and this may explain in part the difference between the water content figures for the two years (54 and 81%).

A better explanation may be found in the very dry winter of 1930–31, a winter which left the water levels of rivers and lakes in many parts of Quebec at record low marks and which undoubtedly left the soil with poor reserves of water.

Following the fall in water content during the summer months, there is a rise during the autumn which is already in progress just before leaf-fall. The water content during the winter months, however, is not at a maximum, but remains (for birch) in the neighborhood of 80%, the rise to the peak of about 100% occurring in the spring. The reasons for this behavior might seem to be clear. Following leaf-fall (October), transpiration is reduced to a very low level while the roots are still able to supply water, but root activities are brought to a standstill soon after this when the ground becomes frozen. In the spring, as the ground thaws out before leaf opening, the roots are again active and the water content rises. The observed rapid fluctuations in water content during 24 hr., on the other hand, forces one to believe that a day or two of continued root activity could, if other conditions permitted, fill the tree after leaf-fall. However, when the rapid fluctuations are taking place, very great tensions are developed (*i.e.*, the actual amount of gas in the tree is low but may occupy a relatively large volume), while in the autumn months the gas rarely shows much less or much more than atmospheric pressure and is present in much larger amount. It is possible that additional water can enter only if this gas is removed by solution or otherwise.

The easy passage of air into and out of the pneumatic system has been noted by many workers, but it may have little to do with gas in the water conducting system, for in spite of Livingston's statement in his review (95) that ". . . all features of the rise of water in plants are generally cared for if we make the supposition that no vessel segment that has once lost its liquid is ever refilled", and Priestley's opinion as to water vapor in cells, it seems that a large proportion of the water-conducting elements do become gas filled during the summer. This is not an argument against the tension hypothesis, since it is easy to imagine that some of the water columns—presumably those in the widest vessels—rupture, and that gas enters, but would seem to be a matter of fact, for the tree may certainly contain a large volume of gas at or about atmospheric pressure, when no tensions are demon-

strable in the conducting system. Observations referred to above, on bleeding from birch in October 1931, when the water content was only about 80%, are in harmony with this.

How do the vessels refill? If sufficiently high root and/or bleeding pressures can be developed, then the physical side of the question is more or less answered though the origin of the pressures may still be puzzling. As a matter of fact there is no evidence that these pressures are adequate for really lofty trees.

Whatever be the causes of root pressure and bleeding, it would seem that they indicate positive pressures in part (or perhaps all) of the hydrostatic system. James and Baker (81), however, conclude, since exudation may proceed while water in vessels is under tension, that: ". . . the positive hydrostatic pressures associated with the exudation must therefore be limited to the cells with protoplasmic contents either in the wood or elsewhere."

In two papers on the mechanism of root pressure, Priestley (129, 130) stresses the importance of the endodermis and its control of entrance and exit of water and solutes from the stele, while Blackman (10) criticizes some of Priestley's views. Further work is reported by Priestley and North (132). While the anatomical evidence suggests that the endodermis plays a part in control of water and/or solute movement, there is some doubt as to its exact role, especially in view of the fact that in large trees there is no endodermis surrounding the greater part of the conducting system in the root.

There would seem to be an intimate relation between sap concentration and development of positive pressures, and this leads to a consideration of the physiological changes that are concerned with winter rest and spring activity, for sap concentration varies with these. Nearly fifty years ago, Schulz (152) noted the disappearance of starch from evergreens during the winter, and an apparent storage of tannin-like materials, while Suroz (171) considered that starch changes to oil, the starch reappearing when the vegetative season begins. It should be noted that a recent publication by Doyle and Clinch (40) throws doubt on the statement as to oil formation.

Fischer (48) made extensive studies of winter reserve stuffs and divided trees into classes. In hardwoods he recognized eight phases:—

1. Starch maximum in Autumn (from leaf-fall till October-November).
2. Decrease in starch (October-November).
3. Starch minimum (December-January-February).
4. Regeneration of starch in spring (March-early April).
5. A second starch maximum (April).
6. Rapid decrease in starch (April-early May).
7. A second starch minimum (Middle-end of May).
8. Summer starch manufacture (End of May until leaf-fall).

Lidforss (93) began to correlate such changes in evergreens with resistance to winter cold and was followed by Czapek (29), Schellenberg (149) and Leclerc du Sablon (140). The last-named agreed with Schellenberg rather than with

Fischer in believing that starch is transformed into hemicelluloses and not into sugar during the winter months. Fabricius (46) also criticized Fischer's views and Niklewski (118) concluded, since starch content is profoundly affected by temperature while fat changes are not, that ". . . der Prozess der Fettumwandlung kann also nicht direkt mit dem Prozesse der Stärkeumwandlung zusammenhängen." In 1907 Lidforss published a very important paper (94) on evergreens, in which he pointed out that all members of the evergreen flora (he studied 130 species belonging to 40 families) are sugar-rich but starch-free in winter, though most of them are starch plants in summer. The intensive study of cold and drought resistance which followed this paper has resulted in a voluminous literature which is somewhat outside the scope of the present discussion. Preston and Phillips (128) concluded that Fischer's classification into hardwood-starch and softwood-fat trees is hardly justified. Sinnott (156) attempted to analyze the factors governing the character and distribution of reserve foodstuffs and decided that the character of the reserve depends primarily on ease of water access to the storage region and that this may be correlated with the carriage of enzymes in the water. Tuttle (177, 178) attempted to induce experimental change of reserves in *Linnaea borealis* in Alberta, and found that temperature appeared to be of fundamental importance, but with Lewis (92) she found, while investigating *Picea canadensis*, that rhythmic changes are more or less independent of temperature. This seems to be supported by the observations of the same workers on *Pinus Murrayana*, *P. albicaulis*, *Abies lasiocarpa* and *Picea Engelmanii*. Riazantsev (138) found, however, that rearrangement of chloroplasts in spring in the leaves of evergreens depends on temperature rather than on time, while Pojarkova (126) remarked the close connection between starch changes and depth of winter rest.

Is the sudden manifestation of hydrostatic activity associated with root pressure, and is bleeding always and entirely a result of the changes discussed above? Such phenomena as autumnal bleeding of birch are evidence against this. At least two distinct types of bleeding occur, however, and they seem to be fundamentally different. Frey-Wyssling (51) stresses this in a recent paper and distinguishes as "bleeding" the withdrawal of water from uncut cells by the osmotic pressure of the cell sap of the cut cells, which are released from wall-tensions, and as "root pressure" the forcing of water into the vessels through the negative suction force of root parenchyma cells.* Wiegand (199) comes to somewhat similar conclusions and agrees with Jones, Edson and Morse (86) that gas expansion due to temperature changes cannot account for the flow of sap from *Acer*. He regards it as a living cell phenomenon, while bleeding of birch and grape he considers to be "without doubt one of root-pressure."

To explain the refilling of cells with water, Münch (115–117) claims that there is a downward stream of water with osmotically active materials in the bark and that when these dissolved materials are removed at the base of the

See also James and Baker (81) in this connection.

tree, the water is pressed out into the xylem and refills the vessels emptied during the summer. He demonstrated and measured this downward stream by loosening strips of bark from below and collecting the exuded sap, calculating that the translocation stream amounts to about 2½% of the total transpiration stream. Among other things he stresses the very rapid streaming in the sieve tubes as significant. Dixon and Gibbon (35) support Münch since the osmotic pressure of sap of *Fraxinus excelsior* decreases from above downwards. This would cause water to be forced into the xylem below. Curtis and Scofield (28), however, find a gradient in the reverse direction. Lund (99–101) states that ". . . an electric current flows downward in the outer cortex and upward in the wood axis," and that this introduces ". . . a distinct possibility, namely that one of the functions of the continuous electric current which is directed upward in the wood is to supply electrical energy for electro-endosmotic flow of sap in an upward direction in the conducting vessels of the wood.* This suggestion may also apply to a downward flow in the cortex as well as transport across the stem." The observations of Stamm (163, 165) appear to be in accord with these views.

While the evidence for the tension hypothesis is so overwhelming as practically to negative all other theories of sap ascent, it is possible that the mechanisms of Münch and Lund explain the refilling of the conducting tracts.

The next question is that of the times of such seasonal changes as onset of root pressure, cambial activity and the appearance of tensions in the conducting system. Several facts noted in Europe by Th. Hartig (65–67) are of particular interest in the present discussion, namely that while bleeding in hornbeam, oak, poplar, lime, etc. ceases before the buds open (and in poplar even before swelling of the buds is noticeable) in hawthorn it commences with the bursting of the buds and continues until the leaves are one-third of an inch long, and in the dog-rose bleeding may continue until the young shoots reach a length of 1½ in.

That positive pressures commence to be evident at about the same time as cambial activity begins is well known. But which of the two actually begins first? A review of the literature is not very helpful. Hartig (67) found that cambial activity commenced in the youngest twigs, and Schröder (150) found bleeding to commence and finish in the root! Brown (16) studied forest trees in the United States (Ithaca) and found that in young trees growth commenced somewhat below the apical shoot and spread upwards and downwards, while in older trees it began in the crown. Knudson (89) also noted that xylem growth commenced below the apex of the tree. He found that phloem development proceeded some way before xylem formation began, which was confirmed by Cockerham (22). Knudson distinguished between increase by swelling and actual growth—the former often commencing some time before the latter—an observation which suggests that water movement may occur before much cellular activity is evident. It is difficult to agree with the views of Pearsall and Hanby (124), that growth of leaves is associated

*See also Marinesco (109).

with positive hydrostatic pressures, since negative pressures may be found in poplar before buds open, a fact which is in accord with Th. Hartig's work referred to above.

Observations on bleeding, pressures, tensions and water-contents of *Betula*, *Populus* and *Acer* throw some light on the subject. In Quebec, the maple may bleed freely in the depths of winter, provided relatively high temperatures are reached, but neither poplar nor birch have been seen to do this, though birch may bleed after leaf-fall. At 2 p.m. on March 5, 1932,—a bright, sunny day, thawing a little in the sun—rapid flow was observed from the south side of maple, but none at all from birch or poplar. Large gas bubbles were apparent in the maple sap, and the liquid appeared to flow both from above and from below. On March 29, with a sun temperature of 5.5° C., only maple was bleeding. On April 9, when the air temperature reached 10.5° C. in the sun but the ground was still frozen, only maple was bleeding. On April 16 the ground was still frozen and small patches of snow remained from a recent fall, but maple, poplar and birch were all bleeding freely, the last both at breast height and at 20 ft. from the ground. On May 3 (sun temperature 21° C.), birch continued to bleed a little (maple was not tapped, but commercial tapping was long over). On the same day, however, tensions had become noticeable in poplar, even in trees with no expanding catkins. It was not until May 14, when the birch leaves were about $\frac{1}{4}$ in. long, that tensions in that tree were recorded, and by that time the water content of borings had dropped from 96% (May 3) to 85%.

Why should these three trees, growing together, differ so markedly in their behavior? One possibility is that the maple can absorb water before birch and poplar, and this might be due to a deep root system in the first, extending to below the frozen soil layer. The root systems of two trees were investigated with the following results. A maple (about two feet in diameter) had a very extensive root system, confined almost entirely to the top two feet of the soil, while a birch showed extensive root development in the upper two feet but also had many small roots extending down to four feet below the surface. It is clear from this that the maple had no positional advantage over the birch, as far as drawing water from under the frozen layer was concerned, nor could it have taken water from the upper layers more readily than birch, for the upper layers remained frozen long after bleeding commenced.

Why should bleeding cease and tensions develop in poplar before bud opening and while birch still shows positive pressures? If evaporation from the twigs of the former be much more rapid than that from the latter, then the question might be answered. A few experiments suggest that this is the case, but before describing these it is well to consider the work that has been done on the subject of transpiration from twigs in the leafless condition.

Stephen Hales (63) seems to have recognized that air can pass through the lenticels, and since his time they have been regarded by most botanists as provision for aeration of the massive trunk. Eder (42) studied *Sambucus*

and *Aesculus* in the winter condition and claimed that the lenticels remained open, and Wiesner (201) confirmed Eder's observation that the lenticels of *Sambucus*, at least, are not closed in winter. Haberlandt (62), on the other hand, found lenticels more or less closed during April, May and the first half of June. He carried out some experiments which showed that evaporation from open lenticels is considerable. Stahl (161, 162) recognized two types of lenticels—one with "Lockere Füllzellen und dichtere Zwischenstreifen" (*Betula*, etc.), the other with "Dichtere Füllzellen, keine Zwischenstreifen" (*Populus*, etc.). Klebahn (88) found lenticels open in winter, though they were sometimes a little more permeable in summer, and in particular he states that *Betula alba* (of which the paper birch is a variety) is the same summer and winter. In his extensive monograph on lenticels, Devaux (30) states that "Toutefois une fermeture complète peut exister très souvent non seulement en hiver, mais à toute époque de l'année." He decides that the primary function of lenticels is transpiration and that aeration is a secondary function. Finally, Strausbaugh (170) finds that water loss from plum is correlated with the number of lenticels.

Experiments carried out just before leaf opening (when poplar showed tension and birch still bled), in which air was forced through twigs of the two trees, proved inconclusive, though the lenticels of poplar appeared to be more open than those of birch. In other experiments, short cylinders of twig were waxed in various ways and evaporation was measured by loss in weight. The results (Table II) support the contention that evaporation from poplar is much greater than that from birch at the time of development of the first tensions in poplar. It may be significant too, that poplar logs lose water more rapidly than birch.

TABLE II

EVAPORATION FROM BARK AND LENTICELS OF BIRCH AND POPLAR (MAY 1932)

Species, etc.	Treatment	Evaporation in mg. per sq. cm. per hour in laboratory
Birch, diam. 1.50 cm.	Lenticels waxed, bark exposed	Hardly more than from completely waxed samples
	Lenticels exposed, equivalent area of bark waxed	Hardly more than from completely waxed samples
Birch, diam. 2.75 cm.	Lenticels waxed, bark exposed	0.12
	Lenticels exposed, equivalent area of bark waxed	0.17
Poplar, diam. 1.75 cm.	Lenticels waxed, bark exposed	0.22
	Lenticels exposed, equivalent area of bark waxed	0.33
Poplar, diam. 3.5 cm.	Lenticels waxed, bark exposed	0.27
	Lenticels exposed, equivalent area of bark waxed	0.41

We have seen that bleeding coincides for the most part with time of maximum water content. In the hardwoods a decrease in water content occurs after the leaves open and from 40 to 50% of the volume of the tree may be occupied by gas at the end of the summer. The rapid diurnal changes in moisture content, on the other hand, are not accompanied by corresponding fluctuations in gas content, for high tensions are developed and there can be little doubt that any great increase in gas content would seriously interfere with the rapid uptake of water that undoubtedly occurs. Most investigations of leaf water contents are in complete harmony with this (see Livingston and Brown (97) and Lloyd (98)). Clements and Loftfield (21) found diurnal changes in sap concentration of leaves, which may be correlated in part, at least, with water changes, while Herrick (69) finds that osmotic pressure and suction tension values of *Ambrosia* tend to reach maximum daily values between 1 and 3 p.m.

Hendrickson (68), measuring the effects of irrigation on diurnal changes in water content of *Prunus*, found that leaves, twigs and trunk all showed a similar morning decrease and afternoon increase.* The afternoon increase noted here was also evident in the present work. Observations of stomata of birch revealed the interesting fact that they may close at, or even before, mid-day, and this no doubt means that transpiration is then greatly reduced.

Work on seasonal variation in conifers, summarized in the second paper of this series, shows conclusively that if any variation takes place in these trees (at least in Quebec) it is so small as to defy detection by the strip and disc method. This is not in agreement with the work of Robert Hartig who described marked seasonal changes in conifers growing in Germany, nor of Münch, who gives figures (117) suggesting a loss of 10 to 15% of the total water during the summer. The observations of Haasis on diameter changes of Monterey pine and redwood in California, referred to above, also point to a marked decrease in water content during the summer, but MacDougal, Overton and Smith (104) found no following increase in Monterey pine after the end of the growing season.

Some of the work on comparative transpirations during summer and winter of broad-leaved evergreens, conifers and deciduous trees is pertinent to the present discussion. Mer (111) investigated evergreen leaves under moderately severe climatic conditions, and decided that they carry on some photosynthesis during the winter. The work of Kusano (91) and Miyaké (113) in Japan is in agreement. Water must be carried to the leaves if much assimilation is to proceed. Stahl (161, 162)) however, using the cobalt chloride method, concluded that the stomata of evergreens, such as *Buxus*, *Mahonia* and *Taxus baccata* are closed by the end of October, which would imply an almost complete cessation of winter activity. Burgerstein (19) gives the following figures showing the effects of temperature changes on transpiration from *Taxus* twigs in winter: If transpiration is represented as

**Linford, however, finds pineapple leaves thinnest (and driest?) in early morning, and thickest between noon and 4 p.m. (Abstract in Am. J. Botany, 21 : 708-709. 1934).*

95 at 12° C., it is 29 at −2° C., 13 at −5.2° and 2 at −10.7 ° C. Ehlers (43), working with leaves of *Pinus laricio austriaca* in Michigan found leaf temperatures considerably above air temperatures, so that measurements of air temperatures may give an exaggerated idea of the severity of the conditions under which the leaves are functioning. Zacharowa (204), however, registered very small differences for *Pinus sylvestris*—less than 1° C. at an air temperature of about −11° C.—and found that gas exchange of *Pinus sylvestris* and *Picea excelsa* is appreciable at quite low temperatures.

In spite of this far from negligible gas movement, Weaver and Mogensen (196) state that "Winter transpiration losses from conifers are scarcely greater than those from defoliated stems of broad-leaved trees." Iwanoff (80) goes further and maintains that the needles of conifers are better protected against loss of water than are hibernating one-year-old twigs of deciduous trees, while Meyer (112) claims that the leaves of pitch pine do not change in water content during the winter. Von Höhnel (70, 71) gives comparative figures also for the vegetative periods of both evergreen and deciduous trees, and shows that, with the exception of larch which transpires rapidly in summer, the former have only a fraction of the water consumption of the latter. Seasonal figures certainly support the contention that transpiration of conifers does not greatly exceed absorption at any time. Yet another point that fits in with this idea is the general absence of bleeding in the conifers, though MacDougal (102) records slight exudation pressures in *Pinus radiata*.

Movement of Water During Seasoning and Flotation

It is convenient now to consider briefly the problem of sinkage with reference to the results summarized above and in the preceding paper (55). The distribution of wood, water, and gas in some of the species studied is tabulated in Table III, so that some idea of the initial flotability of fresh-cut logs may be obtained.

It is evident from this that flotation of poplar and birch cut in late winter or early spring is impossible without seasoning. The same trees cut in late summer, however, may have average densities as low as 0.69 and 0.77 respectively, giving a good margin of flotation.

In summer-cut birch and poplar, the size of the tree, or the position (top, middle, butt) of the log, will have little influence on the density, for outer rings and inner have virtually the same water content at the end of the summer. If winter-cut, the lower parts—and older trees—of poplar will have a margin over upper and younger material by virtue of the relatively drier, and hence lighter, heartwood.

The flotability of softwoods will not vary with the season of cutting, but it will vary with the size of the tree and the position of the log, since the heartwood is always much drier than the sapwood and is present in relatively greater volume in larger logs. This is true even in the case of balsam, since the characteristic wet patches usually occupy so small a percentage of the heartwood that the average density of that part of the log is almost always well below 1.0.

TABLE III

WATER, WOOD, GAS AND DENSITY OF WOODS, AS CUT IN 1931

Species	Part of tree and time of cutting	Water, % dry wt.	% fresh volume occupied by			Density of fresh wood
			water	wood	gas	(water = 1)
Birch	Outer rings. May–June	>120	>59	33	< 8	>1.10
	Outer rings. July–August	50	24	33	43	0.76
	Whole tree. May–June	97	47	33	20	0.98
	Whole tree. July–August	54	26	33	41	0.77
Poplar	Outer rings. May	>160	>67	27	< 6	>1.10
	Outer rings September	70	29	27	44	0.71
	Heartwood	ca. 70	29	27	44	0.71
	Whole tree. May	125	53	27	20	0.95
	Whole tree. September	66	27	27	46	0.69
Jack pine	Sapwood at all times	170	65	25	10	1.04
	Heartwood at all times	35	13	23	64	0.49
Balsam	Sapwood and wet patches of heartwood at all times	200 to 250	62 to 80	20	0 to 18	0.93 to 1.11

Certain points brought out in the preceding paper may be emphasized here.

1. Water can pass relatively freely through the conducting regions when logs are floated (see Scarth and Gibbs (144)), but passes less readily through the heartwood.

2. The loss of water from a log (unless the bark be removed) occurs chiefly from the ends of the log. This is true also of the re-entrance of water, at least in the early stages, when the log is floated, so that log length is very important and end painting may be effective in maintaining flotability.

3. The rate of entry of water is governed by the rate of solution of gas trapped in the log (see Scarth (143)).

4. The top drying of floated (peeled) logs to well below fibre saturation point proves that movement of water in the walls of the wood, in the radial direction at least, may be the limiting factor under some conditions. This is borne out also by the gradients observed in birch logs seasoned for about a year (Scarth and Gibbs, (144) Fig. 1).

5. The gas mixture in the growing tree contains more carbon dioxide and less oxygen than the atmosphere (104). Fermentation may take place in the seasoning or floating log (11, 12) and this results in a still higher concentration of carbon dioxide (144, Table I). Although the carbon dioxide is more soluble than oxygen—2.8 : 1 at 20° C.—the increased volume delays entrance of water, or may even expel it (11, 12; 55, Fig. 11, log 5A).

6. Insofar as the slow currents set up in the log by top drying might assist in solution and escape of gas, top drying, unless very rapid, might actually be detrimental rather than helpful.

When a dry log is first exposed to water, as Scarth (143) points out, entry both of water vapor and liquid water takes place. The cell walls are soon saturated—as volume measurements show—and the air in the cell cavities becomes laden with vapor. After this state has been reached, further entry involves displacement or solution of the air by liquid water. Solution is very gradual; or rather, the diffusion of the dissolved gas is so slow that solution itself is slowed down. It is fortunate for the floater of logs that this is so, since penetration is hindered thereby. But when the matter is approached from the point of view of the mill-men, this solution must be replaced by displacement if even and quick penetration of cooking liquors, etc. is to be attained.

In all these considerations the problem of the passage of water and gas from cell to cell is of the utmost importance, just as it is in the ascent of water in the tree. The difficulty of reconciling the comparatively easy passage of air through bordered pits with the maintenance of water tracts under high tensions has been emphasized above. It crops up again here when measurements of penetrability are made.

Mass flow of water in narrow tubes and in the walls of the cells is fairly well understood, but the question is greatly complicated when air is present. It is possible from observation of the pressures necessary to displace air, and from the permeability of walls to "solutions" containing particles of known size, to get some idea of the diameter of the pores in the walls and pit membranes. The work of Stamm and Bailey has already received notice in this connection. Pore size has been calculated also by Renner (137) and by Frenzel (50). The formula employed is a relatively simple one—used in the calculation of capillary rise in narrow tubes—and substitutes pore radius for tube radius and the pressure necessary to overcome the resistance in the pore (expressed in terms of hydrostatic pressure) for the rise of liquid. We have: $h = \frac{2\gamma}{rsg}$, where h = pressure (expressed as cm. of water), γ = capillary const. = 77 ergs, r = radius of pore in cm., s = density of water = 1, g = 981 dynes. From this it may readily be shown that the pores in the pit membrane are of the order of 0.5–1.0 μ (requiring 3–6 atm. for the displacement of air), while the "intermicellar spaces" of the walls are very much smaller. (Frenzel calculated that the latter are of the order of 1 $\mu\mu$, requiring a pressure of 3000 atm. to force air as such through them.) The contention of Renner,

that the springing of the fern annulus at tensions of about 300 atm. demands a pore diameter of about 10 $\mu\mu$ is surely not justified, for air in solution might pass through at much lower pressures and come out of solution at 300 atm. It is not necessary to postulate mass movement of air as such through the walls in this case. It is thus evident that pore diameter in wood sets a lower limit to the pressures necessary to produce displacement of air. Two obvious steps present themselves at this point if increased penetration be desired: (*a*) Utilization of the natural permeability of wood to the fullest advantage, and (*b*) use of some means to increase effective pore diameter. The first step involves the selection of optimum chip size and shape in mill practice, the second the treatment of the material by physical or chemical means. As a preliminary to (*a*), Saunderson (142) describes the "worst possible" chip shape for sprucewood! The improvement of penetrability under (*b*) may result from an actual increase of pore diameter or from other changes that render effective pores already present but not available for penetration. Little is known of the first possibility, though Sutherland (172) believes that rate of penetration of balsam and red pine, which increases more rapidly with pressure than one would expect, is due to bulging of the pit membranes and consequent increase in pore diameter. The second involves the freeing of adhering tori in heartwood, and need not be discussed here as it has received attention in the first paper of this series (54).

A point of some importance is the curious behavior of sapwood noted by Scarth (unpublished work). When water is forced by ordinary tap pressure in a longitudinal direction through a block, an initial very rapid flow is obtained which falls off after a few minutes. When direction of flow is reversed, it is again rapid, and once more falls away. This might be due to torus action or to blockage of the pores by particles of some sort. Johnston and Maass (85) and Sutherland (172) claim never to have observed torus action in their studies on jack-pine, spruce, etc. but their experiments do not include application of high pressure to sapwood. The observations of Frenzel (50) on the blocking of pits by colloidal gold particles may be an analogous phenomenon—though he makes the curious claim that the particles clog the pits between torus and border on the side away from the pressure. Sutherland (172), who observed similar cessation of flow, thinks it may be due to "Jamin chain" formation.

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A MACHINE FOR TESTING THE RESISTANCE OF PLANTS TO INJURY BY ATMOSPHERIC DROUGHT[1]

BY O. S. AAMODT[2]

Abstract

This machine consists of a glass chamber with a capacity of 40–50 six inch pots through which is forced air which has been heated by thermostatically controlled electric heaters. Dampers and baffles are provided to control the flow of air and to reduce eddies. After exposure for 8–15 hr. at 110° F., 14% relative humidity, and an air velocity of six miles per hour, wheat varieties known to be drought resistant in the field showed less injury from drought than varieties known to be non drought-resistant.

The success of crop improvement programs is usually judged by the degree to which yield and quality have been modified. The pure line theory made it apparent that crops could not be continually improved by selection within a given variety. Elaborate yield tests have resulted in the elimination of varieties markedly affected by certain factors, and the retention of others less affected by these factors. The preferred varieties are not always retained because they possess similar desirable characteristics. The defects in one may be compensated for by desirable characters lacking in others. Information is needed as to why certain varieties perform more satisfactorily than others.

At the beginning of the present century Mendelian methods were introduced generally into the improvement of cereal crops. The mode of inheritance of characters that are readily observable, such as awns, seed color, plant height, maturity, etc., was studied intensively. In many instances simple Mendelian ratios were found. In some cases complicated segregations were obtained and the inheritance of the characters in question was designated as being due to numerous, multiple or probably modifying genes. These early studies, and many present day studies of the same nature, demonstrate that Mendelian principles can be applied to inheritance in cereals, and that a genetic mode of attack is the proper basis for sound crop improvement. Until recent years, however, breeding projects have ignored some of the more pertinent problems in successful crop production. We are sadly lacking in exact information on the mode of inheritance of such important characters as drought resistance, quality, straw strength, winter hardiness, resistance to disease and insect pests, etc. These are the characters that influence directly both yield and quality.

Instead of selecting or breeding for such complex characteristics as yield and quality, it would be more effective to determine the characteristics upon which yield and quality depend. High yield is a character which can be

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determined accurately only over a long period of years. It is not finally determined until the variety has been exposed to all the unfavorable conditions which are likely to occur in the region in which it is to be grown. Fundamental studies on the factors that influence yield are greatly needed. Susceptibility to disease and lack of drought or winter hardiness are recognized as having a direct effect on yield, but the mode of inheritance of the plant's reaction to disease, drought and low temperatures unfortunately is not well known. Knowledge concerning these factors has been limited because of the failure to reproduce them artificially and have them act in a differential manner on the plants being studied. The development of low temperature chambers, artificial disease epidemics, etc., has aided materially in obtaining the desired information. These special methods have contributed greatly to our knowledge of what is wanted in the way of specific and definite varietal characteristics. They have been of great practical value in shortening the period of test required to determine the degree to which a given variety possesses a certain character. Knowledge of why certain varieties perform better than others is of primary importance to the plant breeder. Rapid progress is taking place in the development of laboratory and plot technique for studying the important characters concerned with yield and quality, and in methods of combining the desired characters into a single variety.

A project on breeding for drought resistance in spring wheat was initiated at the University of Alberta in 1929. Soil moisture at Edmonton, Alberta, in most years is sufficient to provide for normal growth and good average yields of grain. Droughts are infrequent, and usually of short duration. Under these conditions there is little opportunity for selecting plants resistant to drought from the segregating hybrid populations. It was evident that either the work must be carried on in a region in which moisture is more of a limiting factor in crop production than it is at Edmonton, or special equipment and laboratory technique would have to be developed for determining artificially the inherent ability of varieties and hybrids to grow and produce grain under conditions of limited moisture.

It is generally recognized that there are two kinds of drought, namely, soil and atmospheric. Soil drought prevails when the soil ceases to provide the plant with sufficient water to take the place of that lost by the leaves in transpiration. The methods for testing plants for differences in ability to withstand soil drought are comparatively simple. Atmospheric drought is caused by hot dry winds which may produce desiccation and killing of the plant tissues even when soil moisture is relatively plentiful. Soil drought is more injurious to the plant, but a combination of both kinds of drought is disastrous. Resistance to drought is usually defined as the capacity of the plant to endure wilting.

A brief description of a special machine for producing artificial drought conditions was received in correspondence with Dr. T. Maximov of the Institute

of Applied Botany and Plant Breeding, Leningrad, Russia, in 1932. The machine was developed for the purpose of testing plants for resistance to "windburn". "The whole thing consists of a glass cage in the middle of which is placed a round table with six holes for pots with plants that turn around on an axis, to allow the plants to have uniform conditions of wind that is blown in with the help of a strong ventilator (fan). Before the air comes into the cage it is heated by means of an oven (they can be of different sort—electrical or heated with oil or wood), which under certain outside conditions very often is sufficient to make it about 16–18% of humidity when it reaches the chamber. Sometimes we also applied an additional drying of the air by means of $CaCl_2$ or by a cold water stream. The wind velocity in our experiment was usually 3 meters per second which, with a temperature of 37–39° C. was just what we needed to provoke an artificial windburn".

The construction of a drought machine with a rotating table, similar to the Russian one, would give a very limited capacity for plant material. In order to be of any great practical value in testing the numerous hybrid strains produced by the plant breeder, the machine should have a capacity of 40–50 six inch pots. A study of the climatic conditions prevalent in the dry area of Alberta during periods when the wheat plants are severely injured by drought indicated that the machine should be able to produce the following conditions: (a) a temperature of 100–120° F.; (b) a wind velocity of 3–10 miles per hour; and (c) a relative humidity of 10–20%. A preliminary report on the construction of the machine was made in 1933 (1).

The type of wind chamber finally decided upon was a glass tunnel 30 in. wide, 10 ft. long, and 40 in. high (Fig. 1). Glass was used in the construction of the chamber in order to provide light conditions as near as possible to normal. One end of the glass chamber was connected by a covered galvanized iron tunnel 20 in. in diameter to the heating units which were placed beneath the floor of the glass tunnel (Fig. 2).

A No. 5 Ventura fan was installed where the glass and galvanized iron tunnels joined. When in operation the fan drew the hot air from the heating units and blew it out through the glass tunnel. It would be undesirable to have the fan motor operating at the high temperature in the tunnel, consequently the short shaft connecting the motor and fan was replaced with one 4 ft. in length. This brought the motor end of the shaft outside the tunnel where it was connected by a pulley and belt drive to the motor (Fig. 2).

With a high air flow it would be impracticable to produce all the heat required with electrical heating units. The machine (which was nicknamed the "chinook" machine) was set up in the greenhouse where the air which was drawn into the electrical heaters could be conditioned easily and cheaply with the regular greenhouse steam heating units. Eight electrical heater coils were made of 16 gauge Nichrome IV heating wire and mounted on

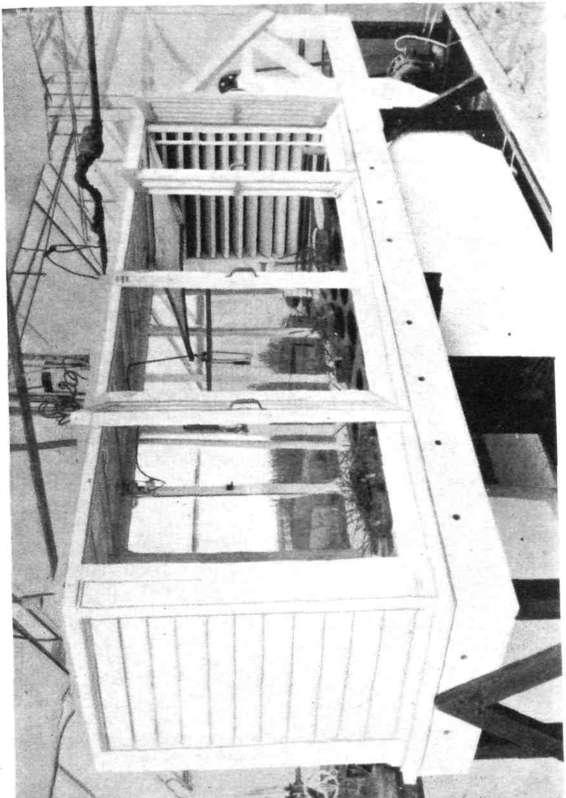

FIG. 1. Front view of the chinook machine showing the glass tunnel with baffle plates in front of the fan and at the end of the tunnel. Air intake and chamber containing electric heating coils shown underneath glass tunnel. Floor of the tunnel shows pots submerged below the path of the hot winds in moist cool sand.

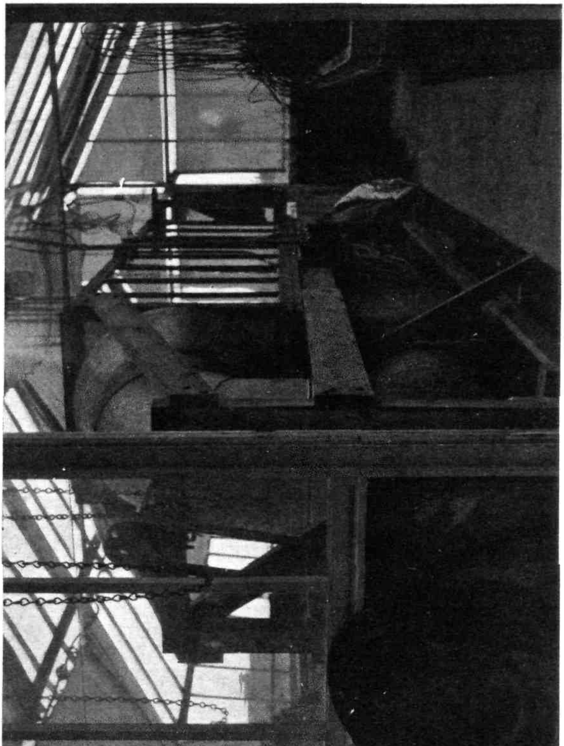

FIG. 2. *Rear view of the chinook machine showing the curved wind tunnel connecting the electrical heating units below to the glass tunnel above. Fan motor is placed outside heating tunnel with four-foot drive shaft and pulley with belt.*

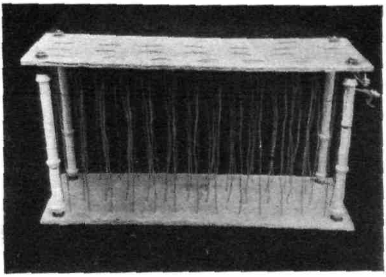

FIG. 3. *One of the electrical heating units consisting of 50 ft. of 16 gauge Nichrome IV heating wire drawn through Transite.*

Transite panels, as shown in Fig. 3. These were placed in a well insulated chamber at the end of the galvanized iron tunnel underneath the glass tunnel. Each of six of the heaters was composed of 50 ft. of wire and each of the two control heaters, of 100 ft. of wire. The coils were arranged so that they could be connected either in series or parallel. The heaters are controlled by four externally operated switches, shown on the side of the machine in Fig. 2. A deKhotinsky bimetallic thermoregulator was placed in the glass tunnel directly in the path of the air flow to give automatic control of the air temperature.

The air flow was controlled by sliding doors at the opening, or air intake end, of the electrical heating chamber, and also by the baffles placed at both ends of the glass tunnel (Fig. 1). The wind velocity was regulated readily within limits at any desired speed by adjusting the sliding doors and baffles, thus causing a greater or less resistance to the flow of air. In the preliminary tests it was discovered that there were several air eddies in the glass tunnel. Small eddies were found in the four corners of the tunnel next to the fan while a large one, about a foot in diameter, extended from the centre of the fan, and tapered off at a point six feet out. A set of baffles placed in front of the fan (Fig. 1) broke up these eddies. This improvement, while causing a reduction of about three miles per hour wind velocity, greatly improved the uniformity of wind distribution in the tunnel. The draw shutters at the exit end of the glass tunnel were also replaced by a set of baffles. Being adjustable they aided considerably in the regulation of the temperature of the air currents.

At first some difficulty was experienced in maintaining a constant temperature of 112° F. for an eight hour period. Three factors were found to be very important in controlling the temperature of the wind passing through the tunnel, *viz.*, the temperature of the greenhouse, the size of the wind inlet aperture, and the size of the outlet aperture. After a few trials it was found that when the temperature of the greenhouse was approximately 85° F., the heating units were able to control the temperature of the tunnel between 110–112° F. for an indefinite period.

Studies were immediately begun on the reaction to atmospheric drought of several drought resistant and non drought-resistant varieties of wheat, which were used as parents in the breeding program. Marked differences were noted in the reaction of these varieties to atmospheric drought (Fig. 4). As soon as a routine technique was worked out, a number of F_4 hybrid lines were exposed, and they likewise showed marked differences in their abilities to withstand injury from atmospheric drought. Resistance to artificially produced atmospheric drought of Milturum 0.321 (drought-resistant), and Selection I-28-60 (non drought-resistant), together with five F_4 hybrid lines,

FIG. 4. *Reaction to atmospheric drought of parental varieties and hybrids of a cross between Milturum (drought-resistant) and Selection I-28-60 (non-drought-resistant). Exposure 15 hr. at a temperature of 110° F., 14% relative humidity and wind velocity 6 m.p.h. Back row: Two pots on left, Milturum. Two pots on right, I-28-60. Front row: Five F_4 hybrid lines. All dead tissue was removed before photographing.*

is shown in Fig. 4. These preliminary results indicated that the chinook machine would be of considerable value in differentiating between varieties and hybrids resistant and non-resistant to atmospheric drought. An intensive study of the whole problem has been undertaken. Results obtained in the laboratory with artificial conditions were checked by growing the material under natural drought conditions in the field. The field experiments are conducted in the drought area at Brooks, in the southeastern part of the province. The details of the experiments conducted, and the results obtained in the laboratory, are being reported in a separate paper by Aamodt and Johnston (2), and the field studies by Aamodt and Torrie (3) and Torrie (4).

Acknowledgments

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A GRAPHICAL STUDY OF THE BLOOD OF NORMAL FOXES[1]

By Arnold H. Kennedy[2]

Abstract

The fluctuations and trends of various cellular elements of normal fox blood are presented in graphical form. The graphs have been prepared from data obtained from a number of foxes of various ages and show the minimum, maximum and mean numbers of blood elements occurring in both males and females in each age group.

The red blood cells, haemoglobin, and to a lesser extent the neutrophiles, on the one hand, have trends of a similar nature and appear to be related. On the other hand the total white blood cells, lymphocytes, monocytes and basophiles also appear to be closely related. A comprehensive picture of the field tends to divide the numbers of blood elements composing the blood of foxes into these two divisions.

The trends and fluctuations, for the same age groups, of the total white blood cells and lymphocytes are almost identical. The monocytes and basophiles also show close similarity. A close similarity in general trend, with less marked fluctuations, exists in the four groups, total white blood cells, lymphocytes, monocytes and basophiles.

Introduction

In order to present the fluctuations and trends of the various cellular elements of normal fox blood, graphs have been prepared. These graphs, based on data from foxes of various ages, are presented in this paper.

Although it is fully appreciated that blood examinations made from a group of foxes, of nearly the same age, and bled at regular intervals, would give a more regular and uniform graph, it was thought that more useful comparative information on the trends of numbers of blood elements could be obtained from blood examinations of healthy, normal foxes grouped according to age.

For this work silver black foxes were divided into groups, according to sex and age. The age groups range from under one month to four years of age and over. An examination was made from each fox in the groups. Each group contained approximately ten male and ten female foxes. Any variation in the cell count in the different age groups was noted and recorded. The maximum, mean and minimum numbers of the various blood elements were determined for each group, and graphs prepared from the data obtained. Some irregular variations due to the effects of chance sampling may be shown in some of the graphs. The standard deviation for the foxes in each age group (Tables I and II) was determined in order that any significant differences between age groups might be noted.

The graphs show at a glance the trend of each blood element throughout the life of a fox.

According to Kennedy (1) the eosinophile is not found in fox blood, but is replaced by a cell which is distinctly basophilic in staining reaction and which is classified as a basophile.

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Contribution from the Ontario Government Experimental Fur Farm, Kirkfield, Ontario.
[2] *Veterinary Pathologist.*

Methods and Technique

Extraction of Blood

The fur was clipped from the integument between two toes and the area cleaned with alcohol, thus exposing the blood vessel located in this region. The vessel was then pricked with the sharp point of a No. 11, Bard Parker scalpel. The first few drops of blood were rejected and then samples were drawn into the pipettes. Standardized pipettes and haemocytometer were used for estimating the cell counts. For the haemoglobin readings, a Sahli haemoglobinometer was used. For the differential count of the leucocytes the blood smears were stained with Hasting's stain and two hundred cells were differentiated under the oil immersion lens.

The Graphs

Figs. 1 to 9 were prepared from the minimum, maximum, and mean numbers of blood elements occurring in both males and females in each age group of foxes. A solid black line was used to designate the numbers of blood elements in males and a broken or interrupted line the numbers in female foxes. The trends or changes occurring over long periods, and the fluctuations, showing the short-period changes, indicate at a glance any increase or decrease, rapid or gradual, that may occur in the number of blood elements at the different stages in the life of the fox. A review of the curves collectively demonstrates the relations of the numbers of the blood elements to one another. Some sets of groups appear to be very closely related while others do not appear to be related.

The standard deviation has been calculated by dividing the sum of the squares of the deviations from the mean by the numbers of cases recorded, and taking the square root of the result. The standard deviations for the foxes comprising each age group are shown in Tables I and II.

TABLE I

STANDARD DEVIATION OF NUMBERS OF RED BLOOD CELLS, GRAMS OF HAEMOGLOBIN, AND COLOR INDEX FOR THE FOXES COMPRISING EACH AGE GROUP

Males				Females			
Age	R.b. cs.	Hb., gm.	Index	Age	R.b. cs.	Hb., gm.	Index
Months				Months			
0– 1	±1.072	±0.65	±0.01	0– 1	±0.825	±0.66	±0.07
1– 2	±0.686	±0.67	±0.07	1– 2	±0.665	±0.53	±0.04
2– 3	±0.636	±0.77	±0.05	2– 3	±0.694	±1.52	±0.09
3– 4	±0.498	±0.38	±0.01	3– 4	±0.753	±0.33	±0.03
4– 6	±1.108	±1.08	±0.03	4– 6	±0.604	±0.76	±0.04
6– 8	±0.977	±0.75	±0.07	6– 8	±0.783	±1.40	±0.06
8–10	±0.492	±0.75	±0.05	8–10	±0.475	±1.05	±0.05
10–12	±1.009	±1.05	±0.05	10–12	±0.832	±0.55	±0.05
12–19	±0.973	±1.19	±0.06	12–19	±1.118	±0.65	±0.07
Years				Years			
2– 3	±1.082	±2.27	±0.08	2– 3	±0.939	±1.50	±0.07
3– 4	±1.308	±1.05	±0.06	3– 4	±1.421	±1.09	±0.05
4– 5	±0.494	±0.41	±0.02	4– 5	±0.483	±1.28	±0.08
				Aged	±0.948	±1.55	±0.07

TABLE II

STANDARD DEVIATION OF THE LYMPHOCYTE, MONOCYTE, NEUTROPHILE AND BASOPHILE COUNTS OF THE FOXES COMPRISING EACH AGE GROUP

Males					Females				
Age	Ly.	Mo.	Ne.	Ba.	Age	Ly.	Mo.	Ne.	Ba.
Months					Months				
0– 1	± 738	±142	±1679	±461	0– 1	± 693	± 67	±1338	±387
1– 2	±1258	±135	±1312	±141	1– 2	±1376	±138	±2090	±228
2– 3	±1995	±157	±1111	±276	2– 3	±1127	± 91	± 654	±161
3– 4	±1565	±149	±1545	±503	3– 4	±1611	±115	±1474	±394
4– 6	±1758	±187	±1825	±211	4– 6	±2063	±162	±1733	±336
6– 8	±1198	±271	±1483	±690	6– 8	±1256	±252	±1079	±224
8–10	±1117	± 97	± 852	±279	8–10	± 904	±111	±1846	±201
10–12	± 762	±144	± 145	±224	10–12	± 936	± 58	±2032	±158
12–19	±1629	±189	±1388	±904	12–19	±1662	±142	±1967	±383
Years					Years				
2– 3	±1592	± 75	±2912	±219	2– 3	±1168	±171	±2520	±491
3– 4	±1850	±139	±2082	±331	3– 4	±1935	± 67	±1457	±340
4– 5	± 564	± 88	± 541	±1297	4– 5	± 708	±286	±1842	±648
					Aged	±1102	± 94	±1052	±194

Fig. 1. Red Blood Cells

The red blood corpuscles vary from month to month in animals of all ages. A steady increase in the numbers of red blood cells was shown in both the male and female fox pups until the age of four to six months. After this age the average increase was not so marked but a slight upward trend continued until the foxes were between ten and twelve months old. After twelve months of age, both male and female foxes showed a reduction in the numbers of red blood cells per cu. mm. A diminution in the average numbers of red blood cells per cu. mm. of blood appears to take place in both male and female foxes as they mature.

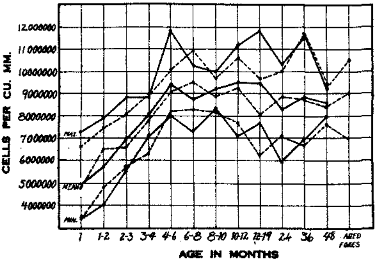

FIG. 1. *Red blood cells. Males* ———. *Females* – – – –.

Figs. 2 and 3. Haemoglobin

Because of the fact that the trends for haemoglobin in the males and the females were so similar, it was found necessary to chart them separately to avoid confusion.

The haemoglobin shows a very marked relation to, and follows very closely, the trends of the red blood cells when the groups are taken as a whole. This is particularly evident in the striking similarity of the haemoglobin curves for the females, although these have greater fluctuations. The amounts of haemoglobin increased with age in both the male and female groups up to ten to twelve months of age. In foxes six to ten months of age, very little increase in the haemoglobin occurred. After this age a rapid increase again took place. A rapid fall in haemoglobin occurred between twelve and nineteen months of age. After this age the haemoglobin increased but decreased again in the adult males at four years of age. As the adult foxes increased in age the haemoglobin in the blood decreased.

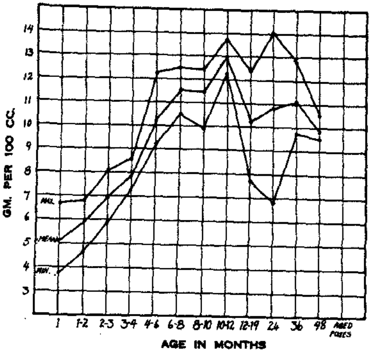

FIG. 2. *Haemoglobin. Males only.*

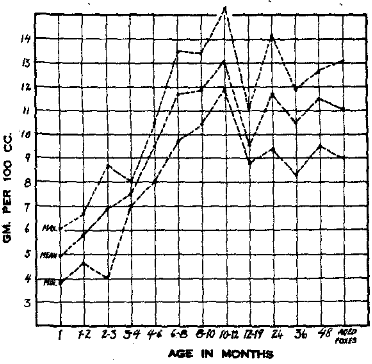

FIG. 3. *Haemoglobin. Females only.*

Fig. 4. Color Index

From under one month of age to between three and four months the color index showed a decline, after which it increased. The highest index reached was found in foxes ten to twelve months old. After this age the index decreased with advance in age of the foxes.

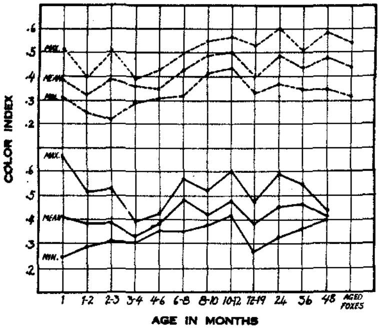

FIG. 4. *Color index. Males ———. Females - - - -.*

Fig. 5. White Blood Cells

The white blood cells show a steady increase up to six months of age, after which a decrease in the numbers takes place. A low level is reached in the females between eight and ten months of age and in the males between ten and twelve months of age. In adult foxes the numbers of white blood cells tended to increase.

Fig. 6. Lymphocytes

The lymphocyte cells showed a steady increase in numbers up to the age of four to six months, after which the numbers commenced to decrease rather rapidly. The decrease continued in the females to the age of eight to ten months and in the males continued to the age of ten to twelve months. The lymphocytes commenced to decrease in the females at an earlier age than in the males and also reached the low level at approximately two months earlier than in the males. After reaching the low level the lymphocytes again showed a rapid increase in numbers, reaching a peak in both males and females between the ages of twelve and nineteen months. From this age on, the lymphocytes decreased in numbers.

Fig. 7. Monocytes

The average number of monocytes per cu. mm. shows very little variation in the groups from young pups to adult foxes. A slight increase occurred in the males between four and six months of age. The highest number reached in the female foxes occurred between six and eight months of age which was a month later than in the males. The numbers decreased in the males between the ages of eight and ten months after which there was a gradual increase in the average number until four years of age. In

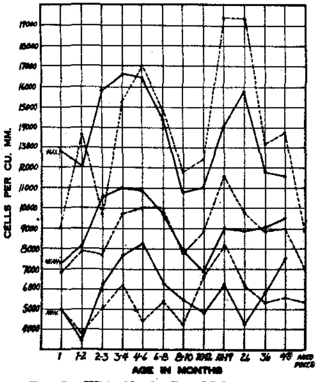

FIG. 5. *White blood cells. Males ———. Females - - - -.*

the females a decrease occurred between the ages of ten and twelve months after which the numbers increased to two years of age. There was a decrease at three, and an increase at four years of age.

Fig. 8. Neutrophiles

The neutrophiles show a relation to the red blood cells. The trend of the neutrophiles does not reach any marked peaks or low levels. There is an upward trend until between three and four months of age. From this age up to between two and three years in both the male and female groups the average neutrophile numbers remained practically at the same level, with a tendency towards a slight increase. The numbers fluctuate slightly above or below 4,500 per cu. mm. After this age the numbers decrease slightly, especially in the female.

Fig. 9. Basophiles

The male foxes showed a decrease in numbers of basophiles at the age of one to two months, after which the number increased. The females continued to show a decrease to the ages between two and three months. In female foxes between four and six months of age an increase in numbers

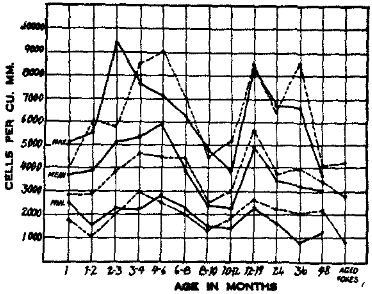

FIG. 6. *Lymphocytes. Males* ———. *Females* – – – –.

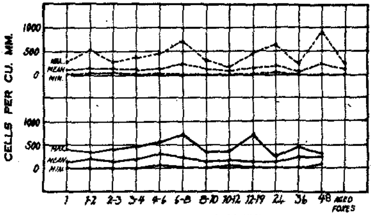

FIG. 7. *Monocytes. Males* ———. *Females* – – – –.

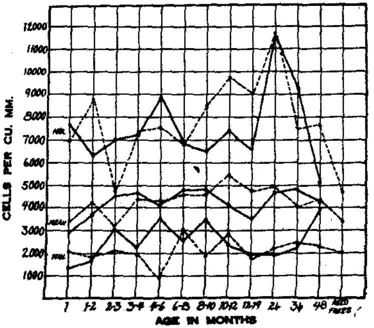

FIG. 8. *Neutrophiles. Males* ———. *Females* – – – –.

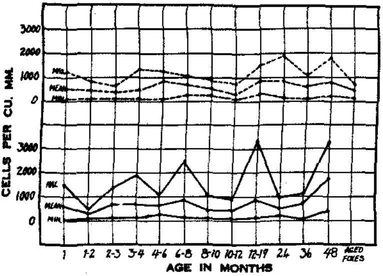

FIG. 9. *Basophiles. Males* ———. *Females* - - - -.

occurred. This was followed by a gradual decrease until ten to twelve months of age was reached. The decrease was followed by an increase in numbers, with slight fluctuations, to the age of four years. In the male foxes between six and eight months of age the average numbers for the group increased. A slight decrease occurred in the average for the group between eight to ten months of age, after which a gradual increase, with slight fluctuations, continued to the age of three years. A marked increase is shown at four years of age.

Discussion

The red blood cells, haemoglobin and to a lesser extent the neutrophiles, on the one hand, have similar trends and appear to be related. The total white blood cells, lymphocytes, monocytes and basophiles, on the other hand, appear to be closely and intimately related. A comprehensive picture of the data collected tends to divide the numbers of elements composing the blood of foxes into these two divisions. Figs. 4 and 5 show that the total numbers of white blood cells and lymphocytes are almost identical in trends and fluctuations for the same age groups, as are also the monocytes and basophiles as indicated by Figs. 6 and 8. Again a close similarity in general trend with less marked fluctuations exists among the four groups, total white blood cells, lymphocytes, monocytes and basophiles. A general observation of their curves shows two well defined peaks divided by a marked depression occurring in foxes eight to twelve months of age. The peaks and depressions are very marked in the total white blood cell and lymphocyte curves and less prominent in the monocyte and basophile curves. In the foxes between eight and twelve months of age the haemoglobin, red blood cells, and to a slight extent the neutrophiles are numerically inversely related to the total numbers of white blood cells, lymphocytes, monocytes and basophiles. The curves for the first group mount to their highest points at eight to twelve months, while those for the other types of cells descend.

Reference

1. KENNEDY, A. H. Studies on the normal blood of foxes. Ontario Department of Game and Fisheries Bulletin No. 6. Toronto. 1933.

NOTE ON THE VARIATIONS IN AREA AND IN STAINING INTENSITY OF RED BLOOD CELLS AND ON THEIR CORRELATION[1]

By Alfred Savage[2], C. H. Goulden[3] and J. M. Isa[4]

Abstract

By means of a laborious technique, based on the use of a projection microscope and a sensitive electric photometer, it has been shown that both the areas and transparencies of stained red blood cells may be determined. From the few observations completed, it appears that, statistically, these attributes are positively correlated in normal human blood and their regression line is straight. *In pernicious anemia the A/T correlation is negative.* Cases of secondary anemia may show either positive or negative A/T correlations. The pathological blood specimens studied all showed non-linear A/T regression lines.

Introduction

In 1931 Savage and Isa (6) reported upon the deflections produced when highly magnified images of red blood cells, stained with acid fuchsine, were projected one at a time into a suitable electric photometer. They demonstrated that, when a series of images were dealt with statistically, the deflections exhibited a greater degree of variation than that shown by the estimated areas of a comparable series. The disagreement was very evident in the case of blood films prepared from anemic patients. By way of explanation, the writers indicated (a) that the images of red blood cells in any series differed visibly in staining intensity as well as in size, (b) that these attributes jointly affected the photometer and (c) that the number of combinations of them which seemed possible would include a wide range. No attempt was made, however, to separate these two attributes.

Scope

This paper describes a method whereby it has been possible to separate and correlate the variations in size and staining intensity of red blood cells. It deals with these properties in the case of normal human blood and touches on them in certain pathological conditions.

General Procedure

The method consisted, essentially, of taking two different observations on each red blood cell of a series. One of these, obtained directly, indicated the area-staining intensity complex. The other was indirect and showed only the area. From these the transparency was derived arithmetically. Figures representing the transparencies and the areas of all the cells in a series were then correlated and subjected to statistical analysis.

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Contribution from the Department of Animal Pathology, University of Manitoba and the Dominion Rust Research Laboratory, Winnipeg, Manitoba, with financial assistance from the National Research Council of Canada.
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Apparatus

The projection microscope and illuminant were those described elsewhere (6, 7), with the addition of a "Watson-Conrady" auxiliary substage condenser. To ensure steadiness, the light was operated by current from a large battery of accumulators and controlled by a suitable rheostat.

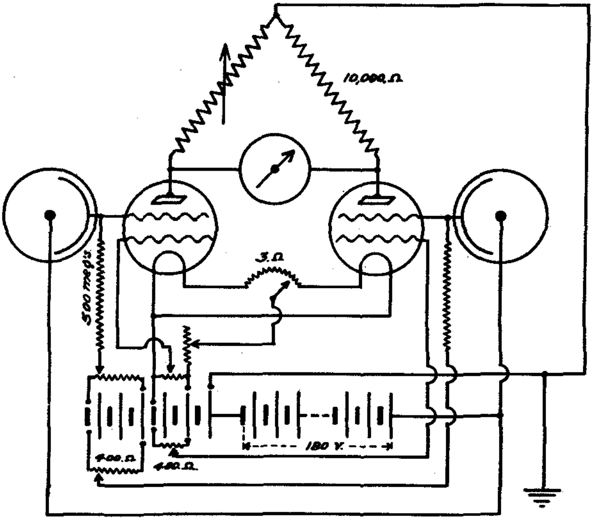

FIG. 1. *The photometer circuit.*

Fig. 1 indicates the photometer circuit. Fundamentally, this was the bridge arrangement devised by Wynne-Williams (8) and modified by DuBridge (2) to suit the FP-54 vacuum tubes of Metcalf and Thompson (3). Both the Burt cells and the valves were very closely matched in all particulars.

By using grid leak resistances of 1000 megohms and a nitrogen filled, tungsten filament lamp there was obtained a calculated galvanometer response of approximately 7000 cm. per lumen. The arrangement was practically free from "drift".

The projection screen was perforated by two circular openings, 2 in. in diameter and 6 in. apart, each of which led to one of the photo cells. They were referred to as the measuring eye and the balancing eye. Between them and perpendicular to the projection surface, an opaque, dull, black screen was erected in such a way that variations in the light admitted to one of the cells was without effect on the other.

Methods

(1) Fixation and Staining of Slides

Having been prepared as thinly as possible in the usual way, the blood films were fixed in absolute methyl alcohol. They were stained in a saturated aqueous solution of acid fuchsine and then washed until no stain could be seen in the plasma between the corpuscles. Rapid drying in air followed.

Unfortunately, because of an exacting prerequisite for this work, namely, that an adequate number of the cells must be quite widely separated, it was not possible to make use of some valuable slides with which the writers had been furnished.

(2) Use of Photometer and Microscope

All the circuits were closed for at least one hour before observations began. This permitted the warming of apparatus indicated elsewhere (7) as necessary. With the photo cells in darkness, the bridge amplifier was then balanced according to the general procedure of Barker and Belchetz (1).

Following this, light was admitted to the measuring eye through the projection microscope (blank field) and an equal amount of light *from the same source* was reflected by a mirror into the balancing eye. This beam did not pass through the microscope: its regulation by a diaphragm afforded a convenient and delicate means of keeping the photometer balanced when illuminated.

Throughout observations the light was adjusted so that the galvanometer response was 150 ($\pm 2.$) mm. for that portion of each blank field which entered the measuring eye.

(3) Measurements of Area and Transparency

a. The area-staining intensity complex. An image of a red blood cell, at 3000 diameters magnification, was focused on the projection screen immediately above the measuring eye. Here its outline was carefully drawn in pencil on a piece of thin, white Bristol board, 2×3 in. The photometer, having been balanced and checked, the image was then moved into the eye and the galvanometer deflection written (in red) on the card beneath the cell outline.

This was repeated until 400 images (and cards) had been dealt with. The procedure was very tiresome and required from seven to nine hours of careful team work on the part of two observers. It should be almost needless to add that the darkroom was *not* lighted during this performance except by the small, carefully shielded lamp, necessary for writing.

b. Area. To render them opaque, the cards were subsequently painted a dull black on the sides which contained no data. Then the outlines were carefully cut out with scissors and, for safe-keeping, each was placed between the pages of a book, together with the stub of the card from which it came.

The comparative areas of the "silhouettes" were obtained by moistening each one slightly and placing it against the glass plate which covered the measuring eye. There it adhered and, because the light conditions were the

same as those which were maintained during the observation of stained cells, the resulting deflection indicated the area of the corpuscle at the magnification employed. As in the previous series of observations, the galvanometer deflections were written on the card stubs but this time *black* ink was used to avoid possibility of confusion.

This part of the work was comparatively rapid. A series of 400 areas could be measured quite easily in three hours.

c. Staining intensity; transparency. The red figure on each card divided by the black one indicated the amount of light which the stained image had cut off, in other words, its average opacity or staining intensity. Conversely, the difference between the black and red figures, divided by the former, showed the average transparency of the image. It is evident that, when added together, these two values equalled unity.

While this form of expression had to suffice for statistical purposes, it was not ideal in that it ignored the manner in which the stainable material was distributed within the cells.

Observations

(1) Normal Blood

Table I shows the data obtained from observations on a sample of normal blood. The horizontal array represents areas, arranged in class intervals of 2 mm. deflection, as shown by the galvanometer. The vertical array indicates the relative transparency, the class interval being 0.029. In this instance the correlation coefficient is +0.4877, a figure which is highly significant.

TABLE I

CORRELATION OF *area* AND *transparency*: 400 NORMAL HUMAN RED BLOOD CELLS, STAINED WITH ACID FUCHSINE.

TRANSPARENCY \ AREA	27–29 (incl.)	30–32 (incl.)	33–35	36–38	39–41	42–44	45–47	48–50	51–53	54–56	57–59	60–62	
0.301–.330 (incl.)			1.	1.									2.
.331–.360													0.
.361–.390		1.	3.	1.	1.			1.					7.
.391–.420	2.		3.	3.	3.	3.	1.						15.
.421–.450			5.	12.	7.	2.							26.
.451–.480		3.	5.	8.	8.	8.	4.						36.
.481–.510	1.	2.	6.	10.	17.	10.	4.	4.					54.
.511–.540		2.	4.	13.	29.	21.	12.	3.	2.				86.
.541–.570			4.	8.	14.	21.	15.	6.	3.				71.
.571–.600			4.	5.	13.	16.	11.	12.	3.	1.			65.
.601–.630			1.	3.	3.	6.	5.	4.	2.	2.	3.		29.
.631–.660				1.	1.	3.	1.	1.				1.	8.
.661–.690													0.
.691–.720										1.			1.
	3.	8.	36.	65.	96.	90.	53.	31.	10.	4.	3.	1.	400.

The strictly linear nature of the regression line A/T is shown by Fig. 2. In plain terms these analyses indicate that, on a slide of normal blood, as ordinarily seen through the microscope, the larger cells appear paler than the smaller ones in definite proportion to their increased size.

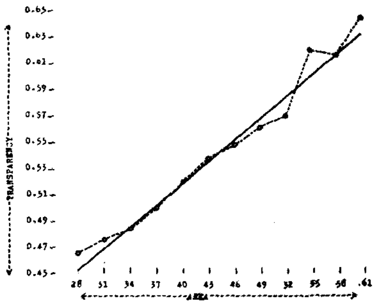

FIG. 2. *Means of tranparency arrays and the regression line of transparency on area: computed from the data of Table I.*

The frequency distribution curves of both area and transparency are essentially normal.

Similar data, obtained from other observations, have confirmed the belief that the example given is typical of normal blood.

(2) *Abnormal Blood*

Primary anemia. Table II gives the data obtained from a slide of blood, considered to have been typical of this condition. The following particulars relate to the case: No. D.3; haemoglobin 76%; red cells 3.65 millions per cu. mm.; color index 1.04.

TABLE II

CORRELATION OF *area* AND *transparency*: 400 RED BLOOD CELLS FROM A CASE OF PRIMARY ANEMIA. STAINED WITH ACID FUCHSINE. AREAS INDICATED AS READ IN MM. OF GALVANOMETER SWING.

TRANSPARENCY \ AREA	36–41 (incl.)	42–47	48–53	54–59	60–65	66–71	72–77	78–83	84–89	90–95	96–101	102–107	
0.654–.670 (incl.)						1.	1.	1.	1.				4.
.671–.687					2.	4.	0.	3.	2.				11.
.688–.704						3.	2.	1.	7.	3.	1.		17.
.705–.721					4.	3.	4.	4.	9.	4.	1.		29.
.722–.738	1.	1.	0.	3.	1.	4.	5.	6.	12.	9.	1.		43.
.739–.755				1.	1.	5.	8.	9.	16.	14.	2.		56.
.756–.722			3.	4.	2.	1.	10.	18.	12.	8.			58.
.773–.789				3.	1.	12.	12.	9.	22.	3.	0.	1.	63.
.790–.806		1.	0.	1.	1.	7.	19.	15.	12.	4.	0.	1.	61.
.807–.823				1.	0.	4.	7.	9.	4.				25.
.824–.840			1.	0.	0.	1.	5.	7.	6.				20.
.841–.857		1.	1.	2.	1.	0.	3.	1.					9.
.858–.874				1.	0.	3.							4.
	1.	3.	5.	16.	13.	48.	76.	83.	103.	45.	5.	2.	400.

In this instance, the correlation of area and transparency is −0.1563. The figure is of more significance because of the negative sign preceding it than because of its size. It indicates that, per unit of area, the larger cells actually stained more deeply than the smaller ones.

The regression line, A/T, is non-linear and suggests heterogeneity.

The frequency distribution curve of the cell areas is negatively skewed and has a high mean value.

Secondary anemia. Case No. D.8; haemoglobin 43%; red blood cells 3.58 millions per cu. mm.; color index 0.61. A correlation surface, dealing with 393 cells from this patient, is shown as Table III.

TABLE III

CORRELATION OF *area* AND *transparency* OF 393 RED BLOOD CELLS FROM A CASE OF SECONDARY ANEMIA. STAINED WITH ACID FUCHSINE. AREAS INDICATED AS READ IN MM. OF GALVANOMETER DEFLECTION.

TRANSPARENCY \ AREA	39–45 (incl.)	46–52	53–59	60–66	67–73	74–80	81–87	88–94	95–101	102–108	109–115	116–122	123–129	
0.730–.746 (incl.)					1.	1.	0.	1.	2.	1.	1.			7.
.747–.763							2.	7.	2.	2.				13.
.764–.780					4.	1.	5.	5.	12.	6.	2.			35.
781–.797			1.	0.	2.	9.	6.	11.	6.	10.	3.	2.		50.
.798–.814				1.	6.	8.	12.	6.	9.	1.	5.			48.
.815–.831	2.	0.	2.	1.	13.	17.	17.	7.	6.	3.	1.			69.
.832–.848		1.	1.	4.	13.	33.	19.	6.	4.	0.	1.	1.		83.
.849–.865				1.	6.	7.	18.	3.	1.	0.	1.			37.
.866–.882	1.	0.	0.	1.	9.	6.	9.	3.	1.					30.
.883–.899					5.	8.	1.	0.	0.	0.	0.	0.	1.	15.
.900–.916					2.	0.	1.	0.	1.					4.
.917–.933							1.	1.						2.
	3.	1.	4.	8.	61.	90.	91.	50.	44.	23.	14.	3.	1.	393.

In this instance, the correlation of area and transparency is −0.3821. The regression line is non-linear. The frequency distribution curve of cell areas is slightly but *positively* skewed and its mean value is high.

Case No. G.1; haemoglobin 15%; red blood cells 1.62 millions per cu. mm.; color index 0.46; anisocytosis very marked. The correlation surface for area and transparency of the stained cells is given as Table IV.

The correlation is +0.4672. Regression line A/T is non-linear. The frequency distribution curve of cell areas is *negatively* skewed and has an approximately normal mean value.

TABLE IV

CORRELATION OF *area* AND *transparency* OF 400 RED BLOOD CELLS FROM A CASE OF SECONDARY ANEMIA, STAINED WITH ACID FUCHSINE. ARRAYS AS IN PRECEDING TABLES.

TRANSPARENCY \ AREA	17–22 (incl.)	23–28	29–34	35–40	41–46	47–52	53–58	59–64	65–70	71–76	77–82	83–88	89–94	95–100	
0.176–.222 (incl.)	1.	0.	0.	1.											2.
.223–.269		2.	.												2.
.270–.316		1.	0.	0.	0.	2.									3.
.317–.363			1.	0.	0.	1.									2.
.364–.410		2.	4.	2.	1.	1.	3.	4.			.				17.
.411–.457	1.	1.	3.	8.	3.	4.	4.	1.	1.	1.					27.
.458–.504	2.	2.	2.	6.	2.	6.	11.	7.	13.	3.					54.
.505–.551		2.	6.	11.	6.	9.	12.	15.	6.	5.	4.	1.			77.
.552–.598		1.	5.	4.	9.	7.	13.	17.	20.	14.	7.	4.	1.		102.
.599–.645		1.	4.	3.	6.	1.	5.	8.	12.	13.	9.	5.	4.	1.	72.
.646–.692			1.	0.	2.	3.	0.	1.	7.	5.	7.	2.			28.
.693–.739				1.	0.	0.	0.	2.	0.	3.	5.	1.	1.		13.
.740–.786				1.											1.
	4.	12.	26.	37.	29.	34.	48.	55.	59.	44.	32.	13.	6.	1	400.

Errors

The greatest source of error was concerned with making the silhouettes. It was a personal one and therefore inconstant. An attempt to assess it was undertaken by making 25 silhouettes of each of a number of blood cells, having different sizes and shapes, and by determining the uniformity of the results. In this way, it was found that, while large, round cells could be reproduced most accurately, small, irregularly shaped ones were troublesome unless great care and patience were exercised. Incidentally, the errors of area affected the figures representing staining intensity. Finally, there were minor inaccuracies, owing to the fact that every apparently blank field between the cells on the blood films did not transmit exactly the same amount of light. Consideration of these factors convinced the writers that the average probable error of their observations amounted to slightly less than $\pm 4\%$.

Discussion

(1) Areas and Area Variations

So far as normal blood is concerned, there is comparatively little to be said under this heading. The example given in Table I shows that the extreme figures representing areas are in about the same relative proportion as the squares of the diameters of the extremes for normal blood cells, given by Savage and Isa (6), *i.e.*,— $(6)^2 : (9)^2 :: 27 : 62$ (approx.). It was a matter of chance that the mean value of the areas, stated in mm. of galvanometer deflection, should have corresponded as closely as it did to the mean area

(of one side) of normal red blood cells in microns (40-42). The chance, however, was a very happy one, because it indicates that *all the figures for area roughly represent square microns.* But these remarks merely show that, compared to an established method, the use of silhouettes and photometer gave reliable results. It is evident that, when only the areas of circular cells are under consideration, the labor involved makes this technique impractical.

In dealing with other than circular cells, however, the method is applicable and diameter measurements fail. Ovoid, sickle-, star- and pear-shaped outlines are matters of indifference to a photometer. For this reason, it is contended that, in the case of grossly distorted cells, full information concerning their areas could be determined by this method.

It may be assumed that curves thus obtained show in *kind* all the variations of the well known Price-Jones (4) curves and do so to a greater degree. This phase of the subject has not been explored more thoroughly for the simple reason that, to the writers, area measurement was not an end in itself: rather it was a necessary step to the determination of staining intensity.

(2) *Staining Intensity and Its Variations*

One objectionable feature of the method was that the degree of saturation to which staining could be continued was fixed by the thickness of the plasma on the slides, and not by the corpuscles. To a slight extent, this must have interfered with the fair comparison of one cell with another on the same slide. As a matter of opinion, it rendered direct comparisons of the staining intensities of cells on different slides highly undesirable. For this reason, measurements of staining intensity in absolute terms were not attempted. But there is nothing to vitiate a comparison of the *trends* of staining in relation to the sizes of the cells, or, to continue with the expression already in use, of the A/T correlations of different specimens of blood.

Normal blood. In the case of normal blood, it has been observed that the regression of transparency on area is linear, and it is fitting that this should be considered in relation to the general law governing light absorption. The rate of the absorption of light passing through the stained blood cells depends on (i) the light source, (ii) the thickness of the cells and (iii) their staining intensity. The first of these being constant, only the two others need be considered.

If either of these factors were constant, the curve resulting from proportional changes in the other would be logarithmic. But the two curves would be in opposite directions. Hence, it seems reasonable to assume that the observed linear relation between area and transparency of a series of stained, normal blood cells is due to a compromise between the effects of thickness and staining intensity. In this compromise the latter effect predominates.

Pathological blood. It may be worth noting that, in relation to the cells of average size, deeper staining of the small cells and/or lighter coloring of the large ones have the same effect on the A/T correlation. They render it positive. Conversely, lighter colored small cells and/or more darkly stained large ones tend to make the correlation negative. There can be little doubt

that the numerical expression of this correlation varies within limits which have not been determined yet in the case of normal blood. For this reason and because of the small number of observations, it is impossible to enter into an extensive discussion of the ways in which the areas and staining intensities of blood cell populations depart from their normal limits during the course of any particular disease, with the possible exception of pernicious anemia. One can merely indicate departures which might take place, leaving it to workers with clinical facilities to determine the facts.

In retrospect, it is not remarkable that the blood cells from a case of typical pernicious anemia should have exhibited a negative A/T correlation. While that condition may not have been previously expressed in mathematical terms, haematologists have long assumed that it existed. The writers have merely demonstrated it by physical means. Incidentally, a negative A/T correlation is not of pathognomic significance. Both positive and negative correlations have been found in cases of "secondary" anemia.

No other general statement is warranted concerning the "secondary" anemias examined. Unfortunately, an example of the microcytic type was not included.

All the pathological bloods exhibited non-linear A/T regression lines.

Summary

It has been shown that the areas of blood cells may be measured almost regardless of the forms in which they occur and that, statistically, the frequency distribution curves of these areas are highly instructive. This is complete extension of the work of Price-Jones (4) and Pijper (5).

The degrees of transparency of the stained individual cells may be obtained also. The transparencies (and staining intensities) of the cells of a series are related to the areas of the same cells either positively or negatively. Theoretically, there seems to be no reason why they might not be quite unrelated under certain pathological conditions.

Without wishing to add to the many systems of classification which have been proposed for pathological blood, the writers suggest that clarification of the subject might result if consideration were given to the area-transparency correlation which they have described.

Acknowledgments

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THE ELECTRODYNAMIC CHARACTERISTICS OF THE QUARTZ PIEZOELECTRIC OSCILLATOR[1]

By James W. Speight[2]

Abstract

The electro-acoustic properties of a piezoelectric oscillator in the form of a quartz–steel sandwich have been studied. The oscillator is assumed to be perfectly efficient and to be emitting divergent waves into the medium in which it is immersed. The arrangement of quartz to steel is investigated with a view to obtaining the best operating conditions of the oscillator. When the radius of the oscillator is infinite, the results reduce to those obtained when plane waves are emitted into the outside medium.

Section 1. Introduction

The general principles of construction of piezoelectric oscillators are briefly described in various textbooks on sound (9). The oscillator considered in the present paper consists of a disc of quartz placed between two steel plates of equal thickness, the outer surfaces of which are in contact with the same medium, so that radiation occurs equally on the two sides of the plane of symmetry.

The object of this paper is to discuss the electro-acoustic properties of such an oscillator. It is evident that the radiation field on either side of the plane of symmetry is equivalent to that generated by a piston surrounded by an infinite, rigid flange. In a number of recent papers by Biquard (1), amplifying lectures given by Langevin at the "Collège de France" (1923), the electro-acoustic properties of quartz–steel "sandwiches" are discussed at length. These calculations, however, refer to infinite radiating surfaces giving rise to plane waves in the medium. Although the characteristics so described may be applied to an actual transmitter of circumference very large compared with the wave-length, it is of some interest to determine the electro-acoustic characteristics of a transmitter of finite radius.

An exact treatment of the properties of the radiation field have been worked out by King (4), so that it is now possible to determine theoretically the acoustic output of a transmitter as described above. For a given radius and frequency, this will obviously depend on the thickness of quartz and steel plates, resonance in the latter being largely responsible for a high output, and the aim of this paper is to determine as closely as possible the optimum output and resonance characteristics. This is all the more important inasmuch as King (4) has shown that, owing to the viscosity of water, there is an optimum range of transmission, depending on the radius of the transmitter, requiring frequencies which make the ratio of circumference to wave-length comparatively small (about 5 or 6). In these circumstances, there is also an optimum radius for the transmitter, the determination of which is of

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considerable interest to the designer. In order to simplify the problem, adial modes of vibration in the quartz–steel sandwich are not considered, although a reference to Section 8 of King's paper shows that there is a rapid fluctuation of pressure over a radiating piston which would undoubtedly set up radial, flexural and compressive waves in the steel disc. The mathematical consideration of these waves is likely to be extremely difficult and is beyond the scope of the present paper. We therefore imagine the surface of the steel plate in contact with water to be covered by a thin, perfectly rigid disc of equal radius, so that the elastic waves in quartz and steel are in one dimension only. Also the dissipation of energy in quartz and steel is neglected, although the inclusion of this factor could, if necessary, be taken into account.

Section 2. Theory

The conditions of the problem are:—

1. The central plane of the quartz disc undergoes no displacement and is selected as the plane of reference $z=0$.
2. The pressures at the surfaces of separation are equal and continuous.
3. The displacements at the surfaces of separation are continuous.

The arrangement of the oscillator is shown in Fig. 1. The radial oscillations are not considered in the steel and quartz, and the energy dissipation is assumed negligible. The wave motion is of the type (2, 7)

$$\frac{\partial^2 \zeta}{\partial z^2} = \rho\alpha \frac{\partial^2 \zeta}{\partial t^2} = \frac{1}{c^2}\frac{\partial^2 \zeta}{\partial t^2},$$

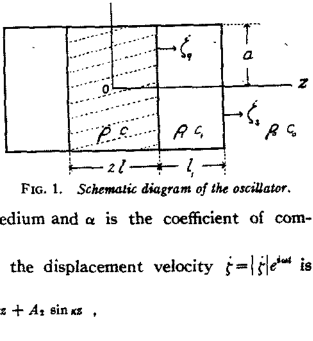

FIG. 1. *Schematic diagram of the oscillator.*

where c is the velocity of propagation, ρ is the density of the medium and α is the coefficient of compressibility.

In the problem dealt with here, the displacement velocity $\dot{\zeta}=|\dot{\zeta}|e^{i\omega t}$ is desired. The general solution is

$$\dot{\zeta} = A_1 \cos \kappa z + A_2 \sin \kappa z \ ,$$

in which $\kappa=\omega/c=2\pi/\lambda$.

In order to satisfy condition 1, the solution must be

$$\dot{\zeta} = A_2 \sin \kappa z \ .$$

If $\dot{\zeta}_q$ is the velocity at any time at $z=l$, and $\epsilon=\kappa l$,

$$A_2 = \dot{\zeta}_q \operatorname{cosec} \epsilon \ .$$

The appropriate solution for the quartz is

$$\dot{\zeta} = \dot{\zeta}_q \operatorname{cosec} \epsilon \sin \kappa z \ . \tag{1}$$

Let the quantities referring to steel be denoted by the subscript 1. Then the solution for the steel has the form

$$\dot{\zeta}_1 = B_1 \sin \kappa_1(z-l) + B_2 \sin \kappa_1(z-l-l_1) \ .$$

If $\dot{\zeta}_s$ is the velocity at the surface of the steel at any time, condition 3 requires that

$$\dot{\zeta}_1 = \dot{\zeta}_q \text{ at } z = l ;$$
$$\dot{\zeta}_1 = \dot{\zeta}_s \text{ at } z = l + l_1 .$$

The coefficients are given by

$$B_1 = \dot{\zeta}_s \operatorname{cosec} \epsilon_1 ,$$
$$B_2 = - \dot{\zeta}_q \operatorname{cosec} \epsilon_1 , \quad \text{where } \epsilon_1 = \kappa_1 l_1 .$$

Hence, the solution for the steel is

$$\dot{\zeta}_1 = \dot{\zeta}_s \operatorname{cosec} \epsilon_1 \sin \kappa_1(z-l) - \dot{\zeta}_q \operatorname{cosec} \epsilon_1 \sin \kappa_1(z-l-l_1) . \tag{2}$$

After differentiation with respect to the time, the continuity of pressures at $z = l$ gives

$$- \frac{1}{\alpha}\frac{\partial \dot{\zeta}}{\partial z} + i\omega\overline{\omega}_1 = - \frac{1}{\alpha_1}\frac{\partial \dot{\zeta}_1}{\partial z} ,$$

since

$$\overline{\omega}_1 = |\overline{\omega}_1| e^{i\omega t}.$$

That is,

$$- \dot{\zeta}_q \frac{\kappa}{\alpha} \cot \epsilon + i\omega\overline{\omega}_1 = - \frac{\kappa_1}{\alpha_1} (\dot{\zeta}_s \operatorname{cosec} \epsilon_1 - \dot{\zeta}_q \cot \epsilon_1) .$$

If we put $m = \kappa\alpha_1/\kappa_1\alpha$ and $A_o = \omega\overline{\omega}_1\alpha_1/\kappa_1$ and rearrange,

$$- \dot{\zeta}_s \operatorname{cosec} \epsilon_1 + \dot{\zeta}_q(\cot \epsilon_1 + m \cot \epsilon) = i\omega A_o. \tag{3}$$

The factor $\overline{\omega}_1$ is the internal piezoelectric pressure given by (1, p. 115, Equation 27)

$$\overline{\omega}_1 = \frac{Q\delta}{2C\alpha_o l} ,$$

where Q is the total charge on surface of the crystal,
δ is the piezoelectric constant,
C is the capacity at constant thickness,
and α_o is the compressibility at constant field.

Now, at the surface of the steel plate and the medium the longitudinal wave motion is replaced by a divergent wave represented by the velocity potential (4)

$$\phi = a\dot{\zeta}_s \int_o^\infty e^{-\mu(z-l-l_1)} J_o(\lambda r) J_1(\lambda a) \frac{d\lambda}{\mu} ; \quad \mu = (\lambda^2 - \kappa_o^2)^{\frac{1}{2}} .$$

The total pressure on the diaphragm is

$$\begin{aligned} P_o &= \int_o^a \rho_o(\dot{\phi})_{z=l+l_1} 2\pi r \,.\, dr \\ &= 2\pi\rho_o a^2 \ddot{\zeta}_s \int_o^\infty J_1^2(\lambda a) \frac{d\lambda}{\mu\lambda} \\ &= 2\pi\rho_o a^2 \ddot{\zeta}_s M , \end{aligned}$$

where M is a complex number with real component $M_1 = H_1(2\kappa a)/2\kappa^2 a$ and imaginary component $M_2 = (1 - J_1(2\kappa a)/\kappa a)/2\kappa$.

After differentiation with respect to the time, the continuity of pressures at $z = l + l_1$ gives

$$- \frac{\pi a^2}{\alpha_1}\frac{\partial \dot{\zeta}_1}{\partial z} = \dot{P}_o . \tag{4}$$

But, since $\dot{\zeta}_s = |\dot{\zeta}_s| e^{i(\omega t - \theta)}$, $\ddot{\zeta}_s = -\omega^2 \dot{\zeta}_s$. Relation (4) becomes

$$\kappa_1(\dot{\zeta}_s \cot \epsilon_1 - \dot{\zeta}_q \operatorname{cosec} \epsilon_1) = 2\rho_o \alpha_1 \omega^2 M \dot{\zeta}_s \ .$$

If we put $\beta = 2\rho_o \alpha_1 c_1 \omega$ in the above,

$$\dot{\zeta}_s(\cot \epsilon_1 - \beta M) - \dot{\zeta}_q \operatorname{cosec} \epsilon_1 = 0 \ . \qquad (5)$$

Equations (3) and (5) give

$$\dot{\zeta}_s = \frac{i\omega A_o \operatorname{cosec} \epsilon_1}{(\cot \epsilon_1 - \beta M)(\cot \epsilon_1 + m \cot \epsilon) - \operatorname{cosec}^2 \epsilon_1} \ .$$

By using the substitutions

$$N_1 = \sin \epsilon \cos \epsilon_1 + m \cos \epsilon \sin \epsilon_1$$

and

$$N_2 = \sin \epsilon \sin \epsilon_1 - m \cos \epsilon \cos \epsilon_1 \ ,$$

the velocity of the diaphragm is

$$\dot{\zeta}_s = \omega A_o \sin \epsilon / \Delta \ , \qquad (6)$$

where $\Delta = \beta M_2 N_1 + i(N_2 + \beta M_1 N_1)$, with argument θ.

Similarly, the deformation of half the quartz crystal is

$$\dot{\zeta}_q = \omega A_o \Delta_1 \sin \epsilon / \Delta \ , \qquad (7)$$

where $\Delta_1 = \cos \epsilon_1 - \beta(M_1 - iM_2) \sin \epsilon_1$, with argument θ_1.

Section 3. Acoustic Energy-resonance

It is evident from Equation (6) that

$$\dot{\zeta}_s = |\dot{\zeta}_s| e^{i(\omega t - \theta)} \ ,$$

where

$$|\dot{\zeta}_s| = \omega |A_o| \sin \epsilon / |\Delta| \ .$$

The acoustic power is

$$\frac{dW}{dt} = P_o \dot{\zeta}_s = 2\pi \rho_o a^2 \ddot{\zeta}_s \dot{\zeta}_s M \ .$$

When P_o and $\dot{\zeta}_s$ are expressed as real quantities,

$$\frac{dW}{dt} = 2\pi \rho_o a^2 \omega |\dot{\zeta}_s|^2 \{-M_1 \sin(\omega t - \theta) + M_2 \cos(\omega t - \theta)\} \cos(\omega t - \theta) \ .$$

The time average value is

$$\frac{d\overline{W}}{dt} = \pi \rho_o a^2 \omega M_2 |\dot{\zeta}_s|^2 \ . \qquad (8)$$

The maximum acoustic power depends on $|\dot{\zeta}_s|^2$ when the frequency and radius are fixed. If the thickness of the quartz is given, the quantity $|\dot{\zeta}_s|^2$ is a maximum when $|\Delta|^2$ is a minimum with respect to ϵ_1. From Equation (6)

$$|\Delta|^2 = F(\epsilon_1) = \beta^2 N_1^2 (M_1^2 + M_2^2) + N_2^2 + 2\beta M_1 N_1 N_2 \ .$$

The condition for a minimum requires that

$$\frac{dF}{d\epsilon_1} = \beta^2 (M_1^2 + M_2^2) 2N_1 \frac{dN_1}{d\epsilon_1} + 2N_2 \frac{dN_2}{d\epsilon_1} + 2\beta M_1 \left(N_1 \frac{dN_2}{d\epsilon_1} + N_2 \frac{dN_1}{d\epsilon_1} \right)$$
$$= 2\{N_1 N_2 [1 - \beta^2 (M_1^2 + M_2^2)] + \beta M_1 (N_1^2 - N_2^2)\}$$
$$= 0 \ .$$

The value of β is small; e.g., $\rho_o = 1.0$ gm./cc., $c_1 = 4.5 \times 10^5$ cm./sec., $\alpha_1 = 6.25 \times 10^{-13}$ cm.^{2}/dyne, $\omega = 2.5 \times 10^5$ rad./sec., so that

$$\beta = 2\rho_o \alpha_1 c_1 \omega = 0.140 \text{ cm.}^{-1} \ .$$

The values of M_1 and M_2 are found by the use of graphs (5, p. 54, Fig. 17). For $\kappa \geq 1$,

$$k = \frac{1-\beta^2(M_1^2+M_2^2)}{\beta M_1}$$

is a positive quantity. In problems of practical interest, $\kappa > 1$ and there is no advantage in discussing the lower limit which κ may approach.
The condition for resonance becomes

$$N_1^2 + kN_1N_2 - N_2^2 = 0 \ . \tag{9}$$

The equation has two roots, *viz.*,

$$N_1 = \tfrac{1}{2}N_2\{-k \pm (k^2+4)^{\frac{1}{2}}\} \ . \tag{10}$$

The second derivative of $F(\epsilon_1)$ is,

$$\frac{d^2F}{d\epsilon_1^2} = 2\beta M_1[k(N_1^2-N_2^2)-4N_1N_2] \ .$$

For

$$N_1 = \tfrac{1}{2}N_2\{-k-(k^2+4)^{\frac{1}{2}}\} \ ,$$

$$\frac{d^2F}{d\epsilon_1^2} = \beta M_1N_2^2(k^2+4)\{k+(k^2+4)^{\frac{1}{2}}\} = \text{a positive quantity.}$$

Thus, the relation for maximum rate of radiation of energy is

$$N_1 = \tfrac{1}{2}N_2\{-k-(k^2+4)^{\frac{1}{2}}\}$$
$$= -bN_2 \ .$$

Therefore

$$\tan \epsilon_1 = (mb - \tan \epsilon)/(m + b \tan \epsilon) \ . \tag{11}$$

For large values of $a(\kappa a = 2\pi a/\lambda \gg 1)$,

$$H_1(2\kappa a) \sim 2/\pi \ ; \quad 1 - J_1(2\kappa a)/\kappa a \sim 1 \ .$$

Hence

$$M_1 \sim 1/\pi\kappa^2 a \ ; \quad M_2 \sim 1/2\kappa \ .$$

If a becomes infinite, M_1 approaches zero. The quantity k becomes very large and

$$b \sim k \rightarrow \infty \ .$$

In this case

$$\tan \epsilon_1 = m \cot \epsilon \ . \tag{12}$$

Relation (12) is in agreement with the work of Biquard for plane waves in the outside medium. The concept of a disc of infinite radius is equivalent to the emission of plane instead of divergent waves into the medium. The energy function transforms into a function of ϵ which was taken at an arbitrary fixed value. There is then a value of ϵ which will produce the optimum maximum acoustic power.
At resonance, it is easily shown that

$$N_1 = \frac{b \cos \epsilon_1 (\sin^2 \epsilon + m^2 \cos^2 \epsilon)}{m \cos \epsilon + b \sin \epsilon} \ ,$$

$$N_2 = \frac{-\cos \epsilon_1(\sin^2 \epsilon + m^2 \cos^2 \epsilon)}{m \cos \epsilon + b \sin \epsilon} \ .$$

Thence

$$|\Delta|^2 = \frac{\cos^2 \epsilon_1(\sin^2 \epsilon + m^2 \cos^2 \epsilon)^2}{(m \cos \epsilon + b \sin \epsilon)^2}[\beta^2M_2^2b^2 + (\beta M_1b-1)^2] \ .$$

Also,

$$\cos^2 \epsilon_1 = 1/(1+\tan^2 \epsilon_1) = \frac{(m \cos \epsilon + b \sin \epsilon)^2}{(1+b^2)(\sin^2 \epsilon + m^2 \cos^2 \epsilon)} .$$

At resonance, the acoustic power from each surface is

$$\left.\frac{d\overline{W}}{dt}\right]_R = \frac{\pi \rho_o a^2 \omega^3 |A_o|^2 M_2 (1+b^2)}{[\beta^2 M_2^2 b^2 + (\beta M_1 b - 1)^2](1 + m^2 \cot^2 \epsilon)} . \tag{13}$$

Of course, there are two radiating surfaces and the total acoustic output is twice Expression (13).

Numerical Example

$\rho = 2.5$ gm./cc., $\alpha = 2.7 \times 10^{-12}$ cm.^{2}/dyne; $\rho_1 = 7.8$ gm./cc., $\alpha_1 = 6.25 \times 10^{-13}$ cm.^{2}/dyne. For a disc of radius 30 cm. and radiation of wave-length 3.77 cm., $\kappa = 1.67$, $M_1 = 3.8 \times 10^{-3}$; $M_2 = 0.30$. Hence $b \sim k = 1880$, $m = \rho c/\rho_1 c_1 = 0.356$. The general condition of resonance, for $\epsilon = \pi/4$, is

$$\tan \epsilon_1 = (mb-1)/(m+b) = 0.355 ,$$

agreeing with Condition (12),

$$\tan \epsilon_1 = m = 0.356 .$$

Section 4. Electrical Power

The electromotive force across a piezoelectric crystal is given (1, p. 113) by $V = Q/C - 4\pi\delta\zeta_q/K_1\alpha_o$, in which the units are electrostatic.

Since $V = V_o e^{i\omega t}$,

$$I = \dot{Q} = i\omega CV + 4\pi C\delta\dot{\zeta}_q/K_1\alpha_o . \tag{14}$$

The first term is a capacitance effect only. The current due to the capacity is additive, so that the equivalent electrical circuit (8) is a parallel circuit with one branch containing a condenser and the other an impedance. The power factor is zero for a perfect condenser and the electrical power can be associated entirely with the impedance arm.

The total velocity of the quartz surfaces is twice $\dot{\zeta}_q$. Hence the current in the impedance arm is

$$I = \frac{\pi a^2 \delta\omega |A_o| |\Delta_1| \sin \epsilon}{|\Delta| \alpha_o l} e^{i(\omega t + \theta_1 - \theta)} .$$

The time average value of the product of the real parts of I and V gives the electrical power

$$P = \frac{\pi a^2 \delta\omega |A_o| |\Delta_1| V_o \sin \epsilon}{2|\Delta| \alpha_o l} \cos(\theta_1 - \theta) . \tag{15}$$

But

$$|A_o| = \delta Q_o / 2C\alpha_o l \omega \rho_1 c_1 = \delta\beta V_o / 4l\omega^2 \rho_o \alpha_o , \quad \text{using} \quad Q_o = CV_o .$$

The electrical power becomes

$$P = \frac{2\pi a^2 \omega^2 \rho_o |A_o|^2 |\Delta_1| \sin \epsilon}{\beta |\Delta|} \cos(\theta_1 - \theta) .$$

Now,

$$\cos(\theta_1 - \theta) = \cos\theta_1 \cos\theta + \sin\theta_1 \sin\theta \ ,$$
$$= \beta M_2(N_1 \cos\epsilon_1 + N_2 \sin\epsilon_1)/|\Delta|\,|\Delta_1|$$
$$= \beta M_2 \sin\epsilon/|\Delta|\,|\Delta_1| \ .$$

The power input is

$$P = \frac{2\rho_0\pi a^2\omega^3 M_2|A_o|^2 \sin^2\epsilon}{|\Delta|^2} \ . \tag{16}$$

The electrical input is equal to the acoustic output. The oscillator is 100% efficient. This agrees with the hypothesis that no energy is dissipated in the quartz or steel. The actual loss of energy in the quartz is due to two factors, acoustic and electrical dissipation. The dielectric losses in crystals are treated by Joffé (3).

Section 5. Optimum Maximum Power

The condition of optimum maximum power is found by examining the acoustic output at resonance. From the previous section, it is seen that $|A_o|$ is proportional to $1/l$, or, more conveniently $1/\epsilon$. The acoustic power is now proportional (Equation (13)) to

$$1/\epsilon^2(1 + m^2 \cot^2 \epsilon) \ . \tag{17}$$

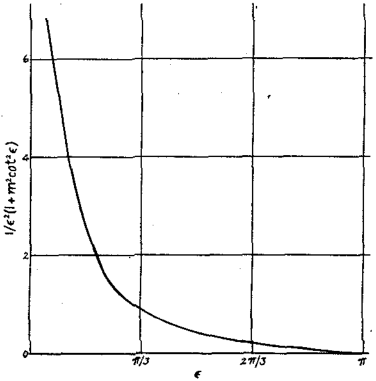

FIG. 2. *Graph showing the rate of acoustic radiation at resonance.*

The function is shown graphically in Fig. 2. There are no maxima in the range 0 to π, but the function approaches infinity for very small values of ϵ.

For $2l \ll \lambda$, ϵ is very small. Function (17) reduces to $1/\epsilon^2(1+m^2/\epsilon^2) \sim 1/m^2$.

However, there is a practical limit to the thickness of the quartz, as it is necessary to select a point on the curve for which the quartz will not break down under the applied voltage. It is also very doubtful whether the quartz crystal will oscillate when the thickness is very small.

There is a critical voltage for a given thickness of quartz, above which the quartz will be punctured. The assumption is made that the voltage amplitude is proportional to the thickness of the quartz, *i.e.*, $V_o \propto \epsilon$.* Since $|A_o|$ is proportional to V_o/ϵ, the output of acoustic energy is proportional

* *The limitations and validity of the assumption may be found in References* (3, 6).

to $1/(1+m^2 \cot^2 \epsilon)$. The maximum rate of acoustic radiation is obtained for

$$\cot \epsilon = 0 \, , \quad \text{or,} \quad \epsilon = \pi/2 \; .$$

The thickness of quartz at this point is

$$2l = \pi c/\omega = 3.14 \times 5 \times 10^5/2.5 \times 10^5$$
$$= 6.3 \text{ cm.}$$

For this case, the thickness of steel is zero as determined by the resonance condition.

Section 6. Summary

1. By the use of the velocity potential ϕ for divergent waves and the displacement velocity $\dot{\xi}$ for plane waves, the velocities $\dot{\xi}_q$ and $\dot{\xi}_s$ at the surfaces of the quartz and steel respectively are obtained.

2. The acoustic power is determined in terms of physical quantities which are known. The theory is valid when the oscillator is immersed in any medium, although the calculations in this paper are those for water.

3. The condition of resonance between the thicknesses of the quartz and steel is found to be

$$\tan \epsilon_1 = (mb - \tan \epsilon)/(m + b \tan \epsilon) \;\; ,$$

which reduces to $\tan \epsilon_1 = m \cot \epsilon$ for a very large radius.

4. The optimum maximum power is produced theoretically when a very thin disc of quartz is used. If one assumes that there is a critical voltage proportional to the thickness of the quartz, the optimum maximum occurs when the quartz is 6.3 cm. thick.

Acknowledgment

The writer wishes to thank Dr. L. V. King who suggested and supervised the problem.

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STARCH CONTENT OF SOME SAMPLES OF CANADIAN WHEAT[1]

BY CLARENCE YARDLEY HOPKINS[2] AND RONALD P. GRAHAM[3]

Abstract

Average samples of seven grades of western Canadian wheat (1933 crop) were analyzed for starch by a polarimetric method. The mean starch content was 51.46%, on a basis of 13.5% moisture. The variation among the grades was small. Protein content varied inversely with starch, but the sum of the two decreased from the higher to the lower grades. The relation of starch content to flour yield is discussed.

Introduction

During a survey of possible industrial uses for Canadian wheat, it was observed that the most likely processes make use of the starch in the grain and that the remaining constituents become by-products. This would be the case in the manufacture of wheat starch, the production of alcohol, or the fermentation to acetone and *n*-butanol.

It was desirable, therefore, to know the starch content of Canadian wheat, not only for the purpose of comparing wheat with other carbohydrate raw materials, but also for the determination of the relative value of the various grades for industrial purposes.

A study of wheat by grades was carried out by Saunders and Shutt in 1908 (11), but the analyses did not include the determination of starch. The only figure for the starch content of Canadian wheat which the authors have been able to find is in a paper by Herd and Kent-Jones (7). They report 51.5% starch in a sample of No. 3 Northern.

There has been a lack of satisfactory methods for the estimation of starch, and this accounts in part for the fact that it is usually omitted from the ordinary analysis of agricultural products. Earlier in the investigation a convenient polarimetric method was perfected (8), and it was used throughout the present study.

The samples of grain were furnished in May 1934 by the Chief Inspector of Grain of the Board of Grain Commissioners at Winnipeg. They were average samples of each of seven grades for the 1933 crop. The sample of Marquis wheat grown in Ontario was supplied by the Cereal Division, Central Experimental Farm, Ottawa.

Experimental

The wheat was cleaned by hand and all weed seeds, foreign grains, straw and hulls were removed. Shriveled and immature wheat kernels were left in the samples.

The grain was ground in a Wiley mill and special precautions were taken to ensure that no material was blown out of the mill. The hopper and the screen were blocked out with paper, and the sample was ground and sifted

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alternately until about 75% passed through a 100 mesh sieve. The oversize was ground by hand in a mortar until only the bran (about 12% of the sample) failed to pass through the 100 mesh sieve. The bran was then mixed thoroughly with the fine material.

Moisture was determined by heating for five hours at 100° C. *in vacuo*. Check determinations were made after the starch analyses had been completed, and the moisture content was found to be virtually unchanged.

The results of the starch determinations are given in Table I.

TABLE I

STARCH CONTENT OF CANADIAN WHEAT

Grade of wheat	Moisture, %	Starch, % (as received)		Starch, % (13.5% moisture basis)
1 Northern	11.15	(53.16, 53.41)	Av. 53.29	Av. 51.88
2 Northern	11.06	(53.49, 53.44)	53.47	52.00
3 Northern	10.70	(51.79, 51.70)	51.75	50.13
4 Northern	10.73	(52.66, 52.66)	52.66	51.03
No. 5 wheat	10.52	(53.81, 53.75)	53.78	51.99
No. 6 wheat	10.56	(53.17, 53.22)	53.20	51.45
Feed wheat	10.37	(53.69, 53.49)	53.59	51.72
				Av. 51.46
Ontario Marquis	10.84	(53.55, 53.82)	53.69	52.08

Weight per measured bushel was determined by weighing a sample of the grain in a graduate as suggested by Aamodt and Torrie (1). A 100 cc. graduate was used, but the agreement between duplicates was not as good as might be desired.

The specific gravity of the kernels was determined by the pycnometer method of Bailey and Thomas (4).

A graphical comparison of specific gravity, starch content and weight per measured bushel is shown in Fig. 1. Schmorl reports that the specific gravity

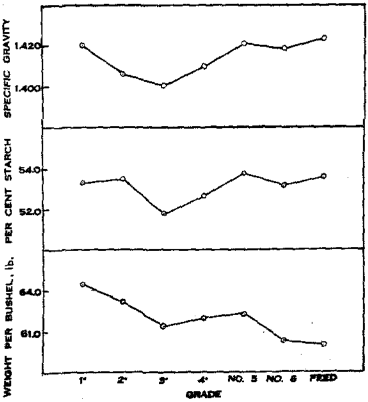

FIG. 1. *Comparison of starch content of wheat with physical properties of the kernels. Moisture basis, as received.*

of wheat varies directly with the starch content (12), and the present results appear to confirm this relation to some extent. However, as Bailey points out, comparisons of this sort should be restricted to wheats of the same type or variety (3).

Protein Content

Protein was determined by the official Kjeldahl method, and the results are plotted in Fig. 2. There is evident a tendency for the content of starch to vary inversely with that of protein. Thus the sample of Grade 3, which has the lowest percentage of starch, is highest in protein.

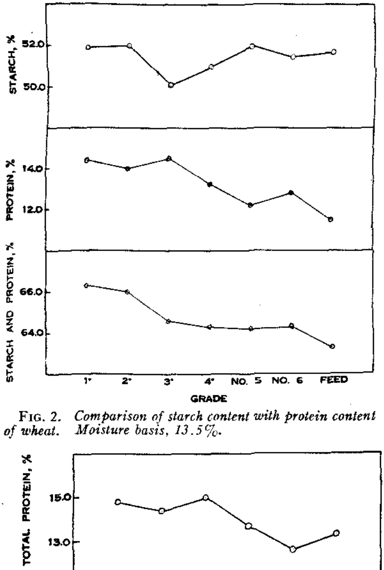

FIG. 2. *Comparison of starch content with protein content of wheat. Moisture basis, 13.5%.*

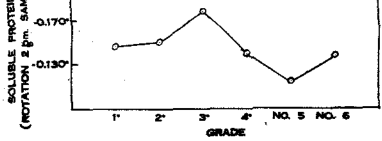

FIG. 3. *Comparison of total protein with protein dissolved during washing with aqueous alcohol. Moisture basis, as received.*

The sum of the percentages of protein and starch, plotted in the lowest section of the graph, decreases to some extent from the higher to the lower grades. The differences are small, however, and this would lead to the conclusion that the feeding value of the lower grades of wheat is almost as high as that of the top grades.

During the starch analyses, the preliminary washing of the ground samples with aqueous alcohol was carried out in identical manner in each case, and the washings were made up to known volume and examined in the polarimeter. Though the optical rotation of the solution is due to several constituents, it is essentially a measure of the amount of dissolved protein. It was found that the amount of dissolved protein deter-

mined in this way varied from grade to grade in the same general way as the amount of total protein. The two are compared graphically in Fig. 3.

Discussion of Results

Comparison with other Analyses of Wheat

The results for starch content are somewhat lower than would be expected from statements in textbooks and elsewhere, which give the starch content of wheat as 60 to 70% (2, 5, 10). This error seems to arise from the fact that carbohydrates as a group are usually determined by difference, and this carbohydrate portion is regarded as consisting almost entirely of starch. In this way the hemicelluloses and related constituents are overlooked. Confusion also arises when the moisture content is not clearly stated.

Canadian hard wheat is relatively high in protein and hence its content of starch may be proportionately lower than that of soft European or southern wheats. This statement is borne out by the results of recent analyses which are shown in Table II.

TABLE II

STARCH CONTENT OF VARIOUS WHEATS

(13.5% moisture basis)

Source	Type	Starch, %	Method	Reference
Western Canada	Hard spring	51.5	Polarimetric	Present work
Western Canada	No. 3 Northern	51.5	Rask	7
United States	—	50.0–58.5	Diastase	9
Argentine	—	51.9	Rask	7
—	Durum	54.0	Rask	7
England	—	54.2	Rask	7
United States	—	56.7	Polarimetric	8
Ontario	Soft winter	59.1	Polarimetric	8

It will be noted that in none of these samples was the starch content as high as 60%.

Starch Content and Flour Yield

The small variation in starch content among the seven grades of Canadian hard wheat was quite unexpected. It was shown definitely by Geddes, Malloch and Larmour (6) that flour yield from wheat of the 1928 crop diminished from Grades 2 to 6, and it was thought that flour yield would be closely related to starch content. Judging from the present work, there is no direct relation. It must be concluded that flour yield depends more on the physical condition of the bran and the resultant ease of separation of bran from endosperm than on the actual amount of endosperm.

The inclusion of the so-called "starchy" kernels in the lower grades may have the effect of raising their average starch content.

It should be noted also that more variation from year to year is to be expected in the lower grades than in Grades 1 to 4, since the definitions of the latter are fixed by statute. The crop of 1933 was of high quality and the wheat in Grades 5, 6 and Feed was consequently of better than average quality.

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THE HYDROGENATION OF ALBERTA COALS

I. PRELIMINARY EXPERIMENTS ON SUSPENSION MEDIA AND CATALYSTS WITH THREE COALS[1]

By E. H. Boomer[2] and A. W. Saddington[3]

Abstract

The action of five suspension media and various catalysts in the hydrogenation of coal has been investigated. It has been shown that the Alberta coals used may be hydrogenated successfully. The properties of the medium have been found to be a controlling factor in the process. The effectiveness of the different media varied with the ease with which they could be hydrogenated and dehydrogenated. Tetrahydronaphthalene was found to be much superior to other media, and showed a greater effect than could be attributed to any of the catalysts used. Experiments showed that this compound was effective because of its action as a hydrogen carrier. Of the catalysts used, molybdic oxide and a mixture of iron and chromium oxides were most effective. The details of the reactions are discussed briefly.

Introduction

The production of oil from coal by reduction with hydrogen at high pressures and temperatures has received considerable attention as offering now or in the future a source of motor and fuel oils. Moreover, the study of the reactions may result in an appreciable extension to the knowledge of the constitution and properties of coal. Reference may be made, for example, to the pioneer work of Berthelot (7), of Fischer and co-workers (11, 12), and to the essential development work of Bergius, Graham and Dunstan (5, 6, 10, 13) leading to commercial application of the process. More recently, a large body of work has been reported covering extensive experiments in Great Britain and Germany, with smaller contributions from numerous other nations (16, 22–26, 30), the literature of which is voluminous and best reviewed in the proceedings of various international coal and power conferences. The general principles of the process and the technique of their application to coal and other carbonaceous material are well known.

The present work is a continuation of investigations dealing with Alberta bitumens as applied to coal, and was undertaken because of the vast resources of low grade coals and the possibility of cheap hydrogen from coal or natural gas in Alberta (2, 3, 4, 18), together with the absence, at present, of adequate petroleum fields. Apart from brief references to the present work (in Reports of the Research Council of Alberta) there appear to have been only two other similar studies on North American coals, one by Beuschlein on United States coals (8) and one by Graham on Alberta coal (15). The necessity for studies on Alberta coals was made apparent by preliminary experiments some years

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ago with coal in a simple autoclave using a mechanical stirrer. These experiments showed the possibility of reduction of Alberta coals, and also showed the necessity of an investigation to determine the optimum conditions of operation, which could not be predicted from other work. The extension of the work beyond a routine test resulted from the belief that the procedure could be improved, and from the discovery that the nature of the suspension medium was as important as that of the catalyst and the coal.

These experiments deal primarily with the best suspension medium and the best catalyst for effecting the greatest conversion of coal to liquids, with the formation of the minimum amount of solid residue. The chemical and physical properties of the oils were considered to be of secondary importance. A second paper will present data of a comparative nature on a graded series of Alberta coals including all ranks. It has been found that with the same coal, whatever the rank, the effect of changing the suspension medium is more pronounced and of more practical interest than that of changing the catalyst, within the limits of the present work. Of the five media used, one, tetrahydronaphthalene, commonly termed tetralin, has been found to be much superior to all others, and produces an increased rate of reaction and yield beyond any effect produced by the catalysts employed. The practical value of tetralin as a medium may not appear great in view of its present cost. It can be largely recovered from the products, however, and moreover is essentially an inexpensive material when prepared in quantity from naphthalene. In addition, naphthalene and related compounds which are readily converted to tetralin and related compounds of nearly equivalent efficiency in the reaction, may be obtained at low cost in the future by pyrolysis of natural gas.

Materials and Apparatus

The three coal samples studied were among those taken in the 1929 Alberta Coal Survey and were made available through the kindness of Prof. E. Stansfield of the Research Council of Alberta. The origin, classification and analysis of these coals as received are shown in Table I. The choice of coals, though somewhat arbitrary, was made on the basis of their probable susceptibility to destructive hydrogenation with the formation of oils. The coals were dried at 125° C. in an inert atmosphere of natural gas at a pressure of 20 mm. of mercury, and were then ground to pass a 100 mesh screen in

TABLE I

CLASSIFICATION AND ANALYSIS OF COAL SAMPLES; ALBERTA COAL SURVEY 1929

Sample No.	419	420	424
Origin	McLeod River Colliery	Pincher Creek—295	Drumheller—678
Canadian classification	Sub-bituminous	Bituminous	Lignite
Moisture, %	6.2	2.2	15.2
Ash, %	10.1	9.2	8.2
Volatile matter, %	38.3	32.5	31.4
Fixed carbon, %	45.4	56.1	45.2
Nitrogen, %	0.6	0.9	
Sulphur, %	0.5	0.8	

a ball mill containing an atmosphere of natural gas. They were stored in stoppered glass bottles.

Five different materials were employed as suspension media in making up coal pastes. The first was a residual oil of I.B.P. 225° C. from the distillation of hydrogenated McMurray bitumen. It was a mixed base oil containing paraffins, naphthenes, traces of olefines and about 15% of asphaltic material. It was highly unstable, cracked at 250 to 275° C., and deposited gums on standing. The second material was crude McMurray bitumen supplied by Dr. K. A. Clark of the Research Council of Alberta. This material was merely dried at 110° C. before use; it contained about 1% mineral matter. The third material was a medicinal paraffin oil of American origin sold under the name Liquid Petrolatum, Heavy, B.P. It was water white, saturated, I.B.P. 300° C.; 95% distilled over at 400° C. without decomposition at atmospheric pressure. The fourth material was phenol, U.S.P. grade, crystals. The last was tetrahydronaphthalene of Eastman practical grade, b.p. 202–204° C. This material will be referred to hereafter by its common name, tetralin.

The hydrogen used was the commercial electrolytic product as ordinarily supplied at 100 atm. in steel cylinders. Occasionally Viking natural gas as supplied locally was used, and also water gas prepared by partial oxidation of natural gas over a nickel catalyst. The Viking gas was 93% methane, the rest being mostly nitrogen with small amounts of ethane and propane. The composition of the water gas was approximately as follows:– Hydrogen, 65.7; carbon monoxide, 32.8; nitrogen, 1.5%. All gases were free from sulphur compounds and, after passage over solid potassium hydroxide, were stored under pressure in steel cylinders connected through appropriate control valves to the autoclave.

Five different catalysts have been used. They were ammonium molybdate, molybdic oxide, chromic oxide, an equimolar mixture of chromic and molybdic oxides, and an equimolar mixture of chromic and ferric oxides. Molybdic oxide was prepared by decomposition of ammonium molybdate at red heat. Chromic oxide and ferric oxide were prepared by precipitation as hydroxides, followed by washing and dehydration. The dry catalysts were ground to pass a 100 mesh screen for incorporation with the coal and suspension medium in the preparation of the coal paste.

The equipment used, including the autoclave, has been described in detail (9). Automatic temperature control and a recording pressure gauge were used. The electrically heated autoclave was machined from 18 : 8 chrome nickel steel and supported in its furnace on a cradle permitting longitudinal rocking to ensure agitation of the contents. In this manner a troublesome packing gland necessary with a mechanical interior stirrer was eliminated and excellent continuous agitation obtained. An appropriate heated expansion valve, charcoal absorber, wet gas meter and water-sealed gas-holder completed the equipment.

Experimental Procedure

A standard procedure was used in all experiments except in regard to the analytical processes to be described.

The charge was prepared from weighed quantities of coal, medium and catalyst. All three were thoroughly mixed together and a weighed quantity of the resulting paste put in the autoclave after which closure was effected and a leak test carried out. Air was washed from the autoclave and tubing by means of hydrogen and the pressure of the hydrogen or other gas raised to the desired initial pressure, usually about 1000 lb. per sq. in. From the volume of the autoclave, paste and fittings, the amount of hydrogen could be calculated readily. The temperature was raised as rapidly as possible with the full power of the furnace to the operating temperature, between 400 and 500° C., and maintained constant for a chosen time. Agitation of the contents by rocking of the autoclave was begun when the temperature was about 100° C.; at this temperature the charge was quite fluid. At the end of the chosen reaction time, the heat was shut off and the autoclave cooled to room temperature or slightly above. Agitation was continued until the temperature was lower than 200° C. Agitation was very necessary at all temperatures where reaction or segregation of the coal was possible, in order to avoid the formation of a hard coke difficult of removal.

Considerable attention was necessary in regard to leaks as the temperature rose, and at the operating temperature. The copper gaskets appeared to yield at about 200° C. and the holding bolts required tightening before this occurred to avoid serious leaks. Again, when operating temperatures of 425° C. and upwards were used, frequent tightening of the holding bolts was necessary in most experiments to prevent the development of large leaks. The softness of copper and its ease of corrosion by sulphur compounds and erosion by liquids and gases at the operating temperatures used led to these difficulties. Another type of closure or the use of some other gasket material, although desirable, was not convenient. The magnitude of the leak was not determinable exactly but was not greater than 10% except rarely, and represented a loss of gases and liquids. It was generally less than the evaporation and mechanical losses incident to emptying the autoclave.

The gases in the autoclave were expanded to atmospheric pressure, passed through activated cocoanut charcoal absorbers, measured by a wet gas meter and stored in a gas holder. After the gas had stood for a time sufficient to reach uniform composition, a sample was withdrawn for analysis in an improved Bureau of Mines apparatus. The gain in weight of the absorbers represented gases and volatile liquids, of which about 30% consisted of butane or lighter constituents and 70%, pentanes and higher hydrocarbons.

The complete process has been termed a cycle. With the same charge of coal, catalyst and medium, the cycle was repeated by adding a fresh lot of hydrogen, and carrying out the heating, cooling and expanding from one to three times in different experiments. Finally, the autoclave was opened and

the oil, water and residue collected as rapidly as possible and kept in a stoppered container. Unavoidable losses of volatile material and some solid residue occurred at this point.

A variety of treatments were devised and examined for use on the material taken from the autoclave. The use of various solvents such as alcohol, ether, carbon disulphide and benzene permitted the separation of various fractions. Such methods were discarded in favor of a simple method producing three products, water, oils and solid residue.

The whole product, solid and liquid, from the autoclave was heated on an oil bath to 125° C. and the distillate condensed by means of an ice condenser. The distillate consisted of water and volatile oils. The water layer was acid and contained hydrogen sulphide in solution, and ammonia when ammonium molybdate was the catalyst. The residue was diluted with ethyl ether and filtered. The residue in the filter was washed with ether until free from soluble material. This solid residue varied in its composition from unchanged coal, in experiments where little action occurred, to a coke-like material that did not show appreciable decomposition on heating, in more successful experiments. It contained all the ash and catalyst. This residue was dried to constant weight and when corrected for the known ash and catalyst content represented unchanged coal. The ether solution of oils was distilled through a short fractionating column in order to recover the ether, the distillation being carried to 300° C. still head temperature. The residues in the flask, though differing widely in viscosity, were classified as pitch. Quantitative analysis was not carried out on the oil fractions. They were invariably unsaturated to some extent and aromatic in character. They distilled without decomposition only when tetralin was the medium and usually deposited red gums on standing.

Results and Discussion

The principal details of a sufficient number of experiments to illustrate the results are shown in tables, supplemented in three cases by graphs of pressure, temperature and time. The tables are largely self-explanatory. However, precise definitions of a few terms may be necessary. The time in hours per cycle represents the time that the autoclave was within 5° C. of the tabulated temperature. This does not represent the total hydrogenation time, as hydrogen absorption began at temperatures near 300° C. The pressures are averages of the values for all the cycles of a particular experiment. The initial and final pressures were measured at room temperature, the maximum pressures at the temperature tabulated. The absorption of hydrogen, yields of products, and loss are shown as percentage by weight of the total charge. The losses include evaporation, mechanical and leakage losses. The gas analyses are expressed in percentage by volume. Data on the yield of carbon monoxide and olefines have been omitted from the table though the amounts were determined in every case. They amounted to 0.1 to 1.5% each in various experiments. The conversion of coal represents the percentage by

weight of the original dry coal, including ash, that was converted to gases and liquids. The figure is based upon the recovered solid residue less catalyst which was the most accurately determined quantity. A degree of uncertainty attaches to this procedure since any coke formed by the medium would be included in this residue. The value may be considered a minimum value. The distillation data give the amounts of the various fractions, including water, boiling below the temperatures tabulated.

Hydrogenations with Complex Hydrocarbon Mixtures

Table II shows results obtained in three different types of experiment using respectively a distillation residue of hydrogenated bitumen, McMurray bitumen and a highly refined paraffin oil.

TABLE II

HYDROGENATION USING VARIOUS SUSPENSION MEDIA AND CATALYSTS

Medium and coal	Distillate from hydrogenated bitumen, Coal 424			McMurray bitumen, Coal 420		Liquid petrolatum, Coal 420	
Experiment no.	94	95	96	131	132	134	137
Coal, gm.	450	450	450	193	183	177	199
Medium, gm.	*550*	*450*	*450*	*208*	*194*	*178*	*212*
Catalyst, 5%	—	—	AM	M	M	M	M
No. of cycles	1	1	2	2	2	2	2
Time, hr./cycle	3	3	3	4	4	4	4
Average temp., ° C.	*425*	*425*	*425*	*425*	*450*	*450*	*425*
Average pressure, lb./sq. in.							
Initial	745	745	720	975	995	1020	1005
Maximum	3585	3240	3195	2265	2430	2480	2195
Final	*930*	*890*	*745*	*505*	*640*	*760*	*600*
Change per cycle	185	145	25	−470	−155	−260	−405
Gas yield, l./kg. of charge	59.8	61.4	127.3	84.5	204	215	76
H_2 absorbed, %	0.1	0.2	0.4	2.0	2.4	2.1	1.7
Charge to liquids, %	—	*14.5*	*13.3*	*56.7*	*22.3*	*22.3*	*48.5*
Charge to solids, %	—	65.7	67.3	29.0	41.8	41.2	33.6
Charge to gas, %	3.4	3.0	5.6	1.3	23.9	12.2	3.8
Charcoal absorber, %	—	—	—	3.1	1.5	2.7	3.5
Loss, %	—	*16.8*	*13.8*	*9.9*	*10.5*	*17.6*	*10.6*
Gas analysis, %							
CO_2	11.8	15.9	13.2	1.6	1.9	0.4	0.6
H_2	33.8	31.1	34.8	69.5	47.9	52.1	73.3
C_2H_6	22.2	18.7	22.3	7.0	24.1	25.8	3.1
CH_4	23.2	16.9	16.4	16.0	15.1	8.9	19.4
Conversion of coal, %				47.1	20.7	24.9	38.2
Liquids, % of charge							
Water	More solid residue was recovered than coal put in and liquid product was small in amount and not distilled.			0.6	2.3	2.4	4.0
Over at 175° C.				10.6	6.8	10.0	10.5
Over at 225° C.				20.2	2.3	13.0	19.8
Over at 300° C.				32.3	13.8	16.5	33.0
Pitch				24.4	8.5	5.1	15.5
Loss (dist.)				0	0	0.7	0

NOTE:—*AM* = $(NH_4)_2MoO_4$; *M* = MoO_3.

Hydrogen absorbed, charge to liquids, charge to solids, charge to gas, charcoal absorber and loss are given as weight per cent of total initial charge.

Experiments 94, 95 and 96 with distillation residues do not constitute an absolutely fair test because the large amount of material reduces the quantity of hydrogen available for reaction. However, a study of the pressure changes

during the experiments showed little reaction, and subsequent examination of the autoclave contents confirmed this conclusion. Fig. 1 shows the pressure–time and pressure–temperature record of Experiment 96. The inflection in Curve B between 300 and 400° C. suggests some reaction, but the very high final pressures and absence of any large pressure drop at 425° C. with time shows such reaction to be slight. The final (cold) pressure was greater than the initial pressure. Virtually all the hydrogen admitted was recovered and considerable coking of the medium occurred. The solid residue weighed more than the original coal added and appeared to be largely unchanged coal mixed with some coke. The high paraffin content of the gases suggests extensive cracking of the medium. An experiment in which pure dry coal and hydrogen alone were used yielded somewhat similar results. No great change in the coal was apparent, no liquids or gases were formed and all the hydrogen was recovered.

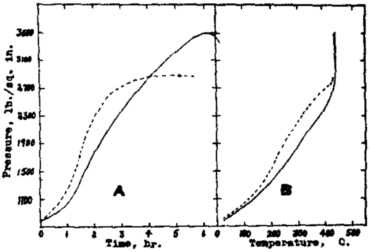

FIG. 1. *Experiment 96. Pressure-time (A) and pressure-temperature (B) relations during hydrogenation at 450° C. with heavy oil as suspension medium.* (——, *Cycle 1*; - - - -, *Cycle 2*).

Experiments 131 and 132, illustrating the use of McMurray bitumen as medium, were more favorable. Absorption of hydrogen, probably by the bitumen, began at 200 to 250° C., and the pressure fell thereafter until, at 425° C., it began to rise slowly. The total drop in pressure during this period of rising temperature was 500 lb. per sq. in. in Experiment 131. The second cycle showed reaction at 300° C., and the pressure began to fall steadily as soon as the constant operating temperature of 425° C. was reached. Experiment 132 at 450° C. was not so satisfactory. The pressure changes were not so favorable and extensive coking took place. It has been shown previously (9) that bitumen becomes highly unstable at temperatures much above 425° C. The coal conversion figure is uncertain and probably much too small.

Experiments 134 and 137 are comparable experiments in which liquid petrolatum was used as medium. The results are related in many respects to those obtained with bitumen. Reaction began at a higher temperature, 300° C. or more, as might be expected from the relative stability of the two media. The rate of hydrogen absorption was greater but the total pressure drop at reaction temperature was less in the case of Experiments 134 and 137. Considerable cracking became evident in Experiment 134 after the first hour at 450° C. Thereafter, the pressure rose rapidly and continuously. The

reduction in oil yield was large and the solid residue must have included much coke derived from the medium. The extent of reduction of coal to oils was as a consequence uncertain.

There would appear to be little difference in effectiveness as media between bitumen and liquid petrolatum at 425°. Coke and gas production was somewhat greater in the case of bitumen but such disadvantages could be controlled by choice of a more suitable catalyst and temperature. The use of liquid petrolatum has certain advantages in laboratory experiments, although its cost would be prohibitive for use on a large scale. The unstable nature of distillates from hydrogenated bitumen rule out their use entirely. It should be pointed out, however, that a stable heavy oil product could be obtained in the hydrogenation of bitumen.

Hydrogenation with Different Gases and Chemical Individuals as Media

The use of phenol as a medium in coal hydrogenation is common (13). Table III shows the results of four such experiments, and Fig. 2 is a graphic picture of the result of Experiment 101. This series showed that phenol is a satisfactory medium for hydrogenation of coal if, as in Experiment 102, an ample supply of hydrogen is used. It is unsatisfactory from the experimental point of view because of the difficulties attached to the treatment of the material recovered from the autoclave. This is contrary to the findings of Graham (13, 14) who has used phenol extensively with satisfactory results. The use of phenol has been condemned, however, by Lush (20) on the ground that it is reduced to cyclohexane. Comparison of data of Table II with those on phenol shows that either bitumen or liquid petrolatum is definitely superior to phenol. With phenol, although the initial reaction temperature was about 350° C., the rate of reaction was much slower than in the other cases. The principal reason for not carrying on further experiments with phenol, however, was the difficulties in regard to treating the products.

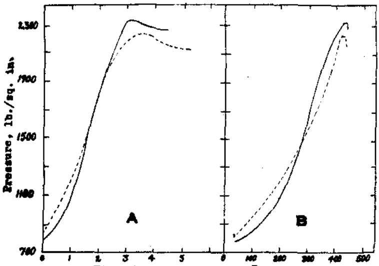

FIG. 2. *Experiment 101. Pressure-time (A) and pressure-temperature (B) relations during hydrogenation at 425° C. with phenol as suspension medium.* (- - - -, *Cycle 1*; ——, *Cycle 2*.)

The remaining four experiments (Table III) were carried out as an investigation of the properties of tetralin. This compound was first used as a suspension medium in the hope that it would be an ideal hydrogenating medium. The ease with which the compound may be dehydrogenated to naphthalene

or hydrogenated to decalin under the conditions of these experiments suggested that it would act on coal as a hydrogen carrier. In addition, its thermal stability and solvent power were favorable properties. A recent investigation by Hall (17) on the naphthalene–tetralin–decalin equilibria with hydrogen under conditions comparable to those of the present work has shown tetralin to be the principal individual product. Less than 10% each of naphthalene and decalin and about 40% of liquids boiling below 195° C. survive after five hours at 450° C. These results are in general agreement with the earlier researches of Spilker (*27, 28*) and Kling (*19*). Experiments whose results are given in Table III and subsequently in Tables IV and V show tetralin to be an excellent medium for the hydrogenation of coal. Experiment 129 was carried out on the assumption that naphthalene would serve as well as tetralin in that it would be hydrogenated to tetralin in the autoclave. It was believed that, with an ample supply of hydrogen, the

TABLE III

REDUCTION USING VARIOUS SUSPENSION MEDIA, CATALYSTS AND GASES

Medium	Phenol				Naphthalene	Tetralin		
Gas	Hydrogen				Hydrogen	Natural gas		Water gas
Experiment no.	98	99	101	102	129	111	113	138
Coal no.	424	424	419	419	419	419	419	419
Coal, gm.	333	355	200	210	200	253	200	197
Medium, gm.	317	355	200	220	125	133	207	123
Catalyst, %	—	5%AM	5%AM	5%AM	4%M	2.5%M	5%M	5%M
No. of cycles	2	2	2	2	2	1	1	2
Time, hr./cycle	3	3.5	4	4	4	3	4	4
Average temperature, ° C.	425	425	425	425	480	450	450	450
Average pressure, lb./sq. in.								
Initial	720	745	790	810	1070	390	480	835
Maximum	2570	2740	2285	2290	2690	1730	1970	2495
Final	665	550	600	550	675	580	720	770
Change per cycle	−55	−95	−190	−260	−395	190	240	−65
Gas yield, l./kg. of charge	71.3	88.4	64.1	95.3	194	29.6	78.0	—
H_2 absorbed, %	1.8	2.0	3.1	2.5	5.1	—	—	1.3
Charge to liquids, %	40.0	37.8	47.6	47.8	37.0	40.8	57.7	42.4
Charge to solids, %	45.4	48.0	41.7	36.6	35.3	43.5	24.3	35.8
Charge to gas, %	6.2	7.9	5.4	8.4	9.3	0.3	1.4	3.9
Charcoal absorber, %	—	—	—	—	1.5	0.8	1.7	3.6
Loss, %	8.4	6.3	5.3	7.2	16.9	14.6	14.9	14.3
Gas analysis, %								CO 14.6
CO_2	15.3	14.8	5.6	5.7	3.1	7.9	5.6	14.2
H_2	43.4	35.1	77.9	63.3	55.7	7.4	8.0	42.8
C_2H_6	5.3	2.9	3.3	6.2	10.6	11.9	7.3	5.2
CH_4	21.5	34.4	10.3	16.1	21.7	62.6	65.2	15.8
Conversion of coal, %	11.4	4.7	22.5	30.9	47.5	35.5	68.0	46.7
Liquids, % charge								
Water	These liquids were separated by fractional solution and were not distilled. They contained much phenol.				5.0	—	—	0.4
Over at 175° C.					10.0	2.6	0.5	3.5
Over at 225° C.					26.4	34.6	37.1	29.7
Over at 300° C.					34.5	39.2	50.9	34.8
Pitch					2.4	1.6	6.8	7.6

NOTE:—$AM = (NH_4)_2MoO_4$; $M = MoO_3$.

same hydrogenation equilibrium would be set up with naphthalene as with tetralin. The result was as expected. A high hydrogen absorption and coal conversion resulted. Only a part of the naphthalene added was recovered in the products of the reactions, together with considerable tetralin. The high maximum pressure was probably due to the greater volatility of naphthalene and tetralin compared to that of other solvents used.

Experiments 111 and 113 were carried out with a view to demonstrating that tetralin was capable of hydrogenating coal in the absence of hydrogen, with the production of naphthalene. The small production of hydrogen suggests a direct transfer of hydrogen from tetralin to coal. The oil contained a great deal of naphthalene and relatively little tetralin. The increase in yield of liquids and conversion of coal resulting from an increase in the proportion of tetralin in Experiment 113 confirms the suggested mechanism of the process. Part of the increase in yield may be due to doubling the amount of catalyst. The oils produced in these experiments appeared to be of a higher quality and thermally more stable than those produced with other media. Experiments 129, 111 and 113 pointed to the conclusion that tetralin was an excellent medium and acted on the coal essentially as a hydrogen carrier as well as a solvent.

The last experiment in Table III, No. 138, was carried out with a definite aim in view, the conservation of hydrogen. The relatively large amount of oxygen in Alberta coals, apart from that held as water, requires considerable hydrogen for its reduction to water. Such water may be considered as waste from the point of view of oil production. Water gas is considerably less costly than hydrogen, and is a reducing gas that should act as effectively as hydrogen on the oxygen of coal. The results obtained in Experiment 138 showed that water gas, with a tetralin medium, was an effective reducing gas. Further, the oxidation of the carbon monoxide by oxygen in the coal occurred to a considerable extent, the ratio of hydrogen to carbon monoxide in the gas rising from 2.0 before to 2.93 after the experiment. The gas produced in both cycles of the experiment had much the same composition. The large production of carbon dioxide and greatly reduced production of water lead to the conclusion that carbon monoxide will remove oxygen from the coal as readily as will hydrogen. Finally, the comparatively good conversion in this experiment with a small hydrogen absorption points to the conclusion that, in other experiments, much of the hydrogen absorbed is used up in the formation of water. This experiment suggests that considerable economies would be effected with a procedure using water gas in one or two cycles, and hydrogen for a final treatment.

Hydrogenations with Tetralin as Medium

The use of tetralin as suspension medium for coal was investigated with the two coals, Nos. 419 and 420. Several of the results are given in Tables IV and V. Some conclusions can be drawn from a consideration of the experiments as a whole.

An immediately noticeable result was the nature of the material in the autoclave after an experiment. The coal, ash and catalyst residue were not in the form of a coke but remained in suspension in the liquids. The oils were thermally stable. The conversion of coal to liquids and gases was high, 91% in one case and frequently more than 80%, although the coals contained 9–10% ash. The gas pressures were never excessively high even at 470° C. Reference to Table IV shows a rough parallelism between the production of water and the number of cycles. This in conjunction with the result, not tabulated, that most of the carbon dioxide produced was liberated in the first cycle, suggests that there are two types of oxygen in the coal. It is probable that much oxygen is combined in carboxyl groups and is liberated by thermal decomposition as carbon dioxide, and that the remainder of the oxygen is combined as hydroxyl groups and removed much less readily by hydrogenation to water. In this connection the results of Experiment 138, Table III, should be considered, as a question arises as to which type of oxygen is reduced by carbon monoxide. It is believed that the increase in carbon dioxide production in this experiment results from reduction of hydroxyl by carbon monoxide. Parr and Hadley (21) have found that carbon dioxide is liberated by thermal treatment of coal. Tropsch (29) believes that the water produced by hydrogenation of coal originates in phenolic bodies.

TABLE IV

HYDROGENATION OF COAL 419 WITH TETRALIN AS MEDIUM AND WITH VARIOUS CATALYSTS

Experiment no.	103	110	112	136	139	140	141
Coal, gm.	200	240	252	200	197	196	213
Tetralin, gm.	200	120	127	150	123	124	131
Catalyst, %	5%AM	5%M	5%M	5%MC	5%FC	5%MC	5%FC
No. of cycles	2	2	4	2	2	1	2
Time, hr./cycle	3.5	4	4	4	4	4	4
Average temperature, ° C.	450	450	450	470	450	425	450
Average pressure, lb./sq. in.							
Initial	965	965	1075	1010	975	1055	945
Maximum	2335	2500	2665	2690	2595	2330	2640
Final	470	585	665	635	585	580	625
Change per cycle	−495	−380	−410	−375	−390	−475	−320
Gas yield, l./kg. of charge	82	146	315	136	98	50	107
H_2 absorbed, %	4.1	3.3	6.1	4.3	3.9	2.4	3.4
Charge to liquids, %	72.5	58.1	55.0	60.0	70.0	64.7	55.5
Charge to solids, %	14.3	21.6	15.8	19.5	13.4	22.5	23.6
Charge to gas, %	3.0	7.6	6.3	9.4	5.6	4.8	9.1
Charcoal absorber, %	—	—	1.0	3.3	3.4	1.8	3.0
Loss, %	10.2	12.7	21.9	7.8	7.6	6.2	8.8
Gas analysis, %							
CO_2	3.9	5.1	2.4	2.8	4.3	6.8	4.3
H_2	70.0	58.9	74.2	64.5	74.5	70.4	70.4
C_2H_6	2.8	12.2	5.8	14.6	4.7	4.7	4.1
CH_4	16.1	16.0	9.7	11.3	12.6	13.5	18.4
Conversion of coal, %	80.0	74.2	83.0	72.0	83.5	70	67.7
Liquids, % of charge							
Water		6.7	11.2	7.1	—	1.8	4.9
Over at 175° C.		18.5	22.4	21.0	6.8	4.3	10.8
Over at 225° C.		28.4	43.5	46.4	38.1	39.7	39.6
Over at 300° C.		48.0	47.0	50.8	47.9	46.8	45.5
Pitch		10.1	8.0	9.2	22.1	17.9	10.0

NOTE:—*AM* = $(NH_4)_2MoO_4$; *M* = MoO_3; *MC* = $MoO_3.Cr_2O_3$; *FC* = $Fe_2O_3.Cr_2O_3$.

TABLE V

HYDROGENATION OF COAL 420 WITH TETRALIN AS MEDIUM AND WITH VARIOUS CATALYSTS

Experiment no.	*119*	*120*	*121*	*135*	*124*	*125*	*126*	*127*
Coal, gm.	200	205	200	200	200	200	200	200
Tetralin, gm.	*109*	*105*	*100*	*165*	*105*	*110*	*135*	*125*
Catalyst, %	5%M	5%M	5%F	6%FC	5%F	5%F	4%FC	7%FC
No. of cycles	1	4	2	2	4	2	2	2
Time, hr./cycle	*1*	*4*	*3*	*4*	*4*	*4*	*4*	*3*
Average temperature, °C.	450	450	450	450	470	470	470	470
Average pressure, lb./sq. in								
Initial	*1235*	*1190*	*1120*	*955*	*1000*	*990*	*1030*	*1060*
Maximum	2625	2820	2455	2385	2510	2405	2290	2570
Final	670	875	765	570	680	625	—	610
Change per cycle	*−565*	*−315*	*−355*	*−385*	*−320*	*−365*	—	*−450*
Gas yield, l./kg. of charge	52	217	123	96	254	140	133	133
H_2 absorbed, %	2.8	5.6	3.3	3.5	5.9	3.6	4.5	4.1
Charge to liquids, %	*66.6*	*59.4*	*56.8*	*75.5*	*32.8*	*48.7*	*40.3*	*55.4*
Charge to solids, %	21.6	16.8	22.8	9.9	30.5	22.6	21.8	18.5
Charge to gas, %	1.7	7.5	5.0	8.3	12.9	7.0	6.6	8.1
Charcoal absorber, %	*2.3*	*1.8*	*1.0*	*0.6*	*1.3*	*0.5*	*0.0*	*0.8*
Loss, %	7.8	14.5	14.4	5.9	22.5	21.2	31.3	17.2
Gas analysis, %								
CO_2	*0.4*	*0.3*	*1.1*	*0.7*	*0.4*	*0.5*	*1.0*	*0.9*
H_2	76.4	80.2	75.8	70.8	74.0	67.3	65.0	68.5
C_2H_6	3.0	4.0	5.5	10.5	6.0	8.5	7.2	10.9
CH_4	*11.6*	*6.8*	*9.6*	*14.1*	*12.8*	*13.3*	*19.0*	*12.7*
Conversion of coal, %	72.5	80.4	71.5	91.0	58.5	71.0	68.2	78.0
Liquids, % of charge								
Over at 175° C.	*10.0*	*13.1*	*3.4*	*13.7*	*11.2*	*8.4*	*9.0*	*9.5*
Over at 225° C.	42.9	42.9	33.4	43.1	25.0	34.1	31.0	32.2
Over at 300° C.	57.3	50.8	39.4	47.8	28.4	37.6	34.4	43.4
Pitch	*9.3*	*8.6*	*17.4*	*27.7*	*4.4*	*11.1*	*5.9*	*12.0*

NOTE:—$M = MoO_3$; $F \approx Fe_2O_3$; $FC = Fe_2O_3.Cr_2O_3$.

A comparison of the data of Tables IV and V from the point of view of the type of coal used does not allow of any certain conclusion being drawn. On the whole, Coal 419 appeared to be more susceptible to hydrogenation, as might be suspected from its classification as sub-bituminous. On the other hand the bituminous coal, No. 420, gave greater yields of oil and less water, and showed the maximum conversion of 91% in Experiment 135 with an iron-chromium catalyst. The effect of a temperature greater than 450° C. was generally adverse, as shown by increased gas yield and increased solid residue. Hall (17) has shown in work on the hydrogenation of naphthalene to tetralin that marked decomposition with the formation of gas and coke occurs at 460° C. or higher. No great increase in coal conversion was obtained by using more than two cycles. Usually a small increase was observed but at the same time the yield of liquid decreased and the gas production increased.

A study of the reaction by following the pressure–time–temperature curves showed some interesting results. Fig. 3 gives the record for one experiment, No. 110, and may be taken as typical. Hydrogen absorption began at about 300° C. with a rising temperature. Distinct evidence of an exothermic reaction was always obtained in these hydrogenation experiments when hydrogen absorption began. The rate of temperature rise showed a sudden

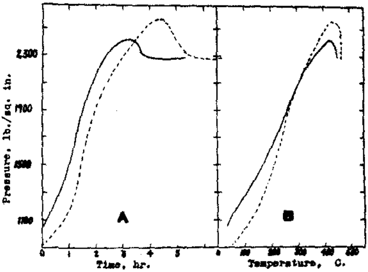

FIG. 3. *Experiment 110. Pressure-time (A) and pressure-temperature (B) relations during hydrogenation at 450° C. with tetralin. (——, Cycle 1; - - - -, Cycle 2.)*

increase in the neighborhood of 300° C. Usually the pressure began to drop before the temperature reached 400° C., and for the first half hour at 450° C. fell with great rapidity. After one to one and one-half hours at 450° C. the pressure became constant. It is believed that hydrogen absorption continues, however, and is balanced more or less by gas production. The second cycle of the experiments showed generally similar results, which were modified as expected by a higher maximum pressure and smaller rate of pressure drop. The relative amounts of coal and tetralin had a definite influence on the pressure developed and on the coal conversion. Increased amounts of tetralin resulted in an increased conversion and decreased maximum pressure. The influence was slight but indicated that at least three parts of tetralin to four of coal should be used for satisfactory operation.

An estimate of the relative value of the various catalytic mixtures is difficult to make from the tabulated data alone and requires consideration of the pressure–time relations. A catalyst may have two important effects; it may promote hydrogenation and it may promote thermal decomposition. Undoubtedly both reactions occur under the experimental conditions and are promoted to some extent by a catalyst. In addition the quantity and quality of the oils produced and the quantity of gas produced depend upon the effect of the catalyst on the two reactions. From the point of view of maximum conversion of coal to liquids, the equimolar mixture of iron and chromium oxides was the best catalyst. Of the others, only molybdic oxide need be considered. This caused smaller total conversion than did the iron–chromium mixture, but had the advantages of producing a superior oil from the point of view of unsaturation and pitch content, and of resulting in a higher rate of hydrogen absorption. Though the iron–chromium mixture promoted liquefaction of the coal it promoted also thermal decomposition to a greater extent than did molybdic oxide. For a systematic investigation, not yet reported, of a number of Alberta coals, molybdic oxide was chosen as the best catalyst.

A few observations regarding the liquid products are of interest. The water obtained on distillation was invariably acid. The acid constituents were not identified except by demonstrating their organic nature and volatility. The oils were different in many respects from those produced with other media. Fractional distillation of the 175–225° C. fraction showed the presence

of a large amount of material boiling between 195 and 215° C., presumably tetralin, naphthalene and decalin, for the most part. The remainder of the oil fraction was chiefly notable by its stability when distilled at atmospheric pressure to 450° C. No decomposition occurred. From 225 to 350° C. the distillate was a heavy yellow fluid of apparently high viscosity. Occasionally, colorless, wax-like crystals separated from this distillate during the later stages of the distillation. At about 350° C. the color of the distillate changed to red and the distillate from the final stages of the distillation became solid or semisolid at room temperature. At 450° C., about 5% of the original coal remained as pitch, which boiled quietly at 450° C. and on cooling solidified at about 200° C.

Summary and Conclusions

These experiments show that the Alberta coals examined are readily hydrogenated to an extent depending largely upon the medium used. A suspension medium is necessary in order to obtain a reasonable rate and yield in the process and the results emphasize the importance of a suitable choice. A heavy residual oil, such as may be obtained from hydrogenated bitumen, was unsatisfactory because of its instability and probable lack of solvent power. Bitumen showed some promise as a medium and was reduced itself, probably more than the coal. Liquid petrolatum appeared to be a fairly effective medium for laboratory purposes, but had the disadvantage of suffering considerable decomposition to coke in the process. Phenol was of some value, chiefly because of its stability, but it had the great disadvantage of precluding a simple procedure for the analysis of the products. Tetralin was much superior to the other media in promoting rapid and extensive liquefaction of the coal.

Roughly speaking, the effectiveness of a medium paralleled its ease of hydrogenation and dehydrogenation. The results suggested that tetralin acted both as a good solvent and as a hydrogen carrier, the latter function overshadowing the solvent action and the effect of any added catalyst. The suggested mechanism is one of transfer of hydrogen from tetralin to the coal compounds and the hydrogenation of the resulting naphthalene to tetralin again by hydrogen. The experiments with tetralin and natural gas and with naphthalene and hydrogen, together with the presence of naphthalene in the oils of Tables IV and V, confirm the hydrogen carrying action. It has been shown elsewhere that tetralin is readily prepared from naphthalene by hydrogenation and is just as readily dehydrogenated (12), and that tetralin is a good hydrogenating agent in the presence of palladium (1). There is an obvious field for further work in the examination of other compounds capable of reversible hydrogenation and stable under the conditions of these experiments.

The maintenance of high hydrogen concentration in the autoclave and effective temperature control between 425 and 450° C. were shown to be optimum operating conditions.

The oxygen content of the coal appeared mostly as carbon dioxide and water. It was suggested that the carbon dioxide arose from decomposition of carboxyl groups and the water from hydrogenation of hydroxyl groups. An experiment in which a mixture of hydrogen and carbon monoxide was used in place of hydrogen showed an apparent reduction of phenolic compounds by carbon monoxide.

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REVIEWS AND NOTES

SENSITIVITY AND OUTPUT OF VARIOUS TYPES OF PHOTOCELLS[1]

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Abstract

When the current sensitivity of a short-circuited photo-emissive cell is defined in the same manner as the grid-plate conductance of a vacuum tube, the ratio microamperes to lumens in modern photo-tubes is of the same order as the ratio milliamperes to volts in vacuum tubes, but a change of one lumen corresponds to virtually the entire range, 1 ft.-candle to 1000 ft.-candles, concerned in problems of illumination. Taking into account the detector action and the retarding voltage across the load used with a barrier film cell, the resistance giving the highest output of power is nearly equal to $(\frac{3}{4}\, klG)^{\frac{1}{3}}$, provided that l be less than 1000 ft.-candles and that the film rectifies according to a square law, Gv^2. It is advisable to develop cells possessing relatively small values of G and an exponent of v not larger than two.

In industrial work, photoelectric cells are often used with an appreciable load in the circuit, consisting, in the simplest case, of a high resistance or of a coil which forms part of a relay or a transformer (1, 4, 6). It has been rightly said that no photocell is better than the circuit in which it is used; on the other hand, though the sensitivity of the best photo-emissive, barrier film or photoconducting cells may be of a similar order, their internal resistance depends on the illumination in a manner which differs considerably from one type to another. Moreover, in the case of the more recent barrier film cell, the load inevitably is in parallel with the internal resistance; in the other cells it is in series with the internal resistance. If a device is to be actuated by a photocell, the question arises—which type of cell accomplishes the task most efficiently? As an illustration, the response of different cells having about the same sensitive area, 1 sq. in. (6.45 sq. cm.), may be compared when they are exposed to an illumination, l, of 10 ft.-candles, the intensity sometimes recommended for offices, factories and showrooms; and also when this value increases or decreases by 10%. The smallest variation which the eye can detect when comparing two fields side by side is about 1%.

For a sensitive surface of 1 sq. in. the mean light flux at 10 ft.-candles is about 0.07 lumen (or between 1/14 and 1/15 lumen). The load may be either a coil of 50,000 turns and a resistance, R, of 10,000 ohms, with a contact which, in order to be brought from the open to the closed position,

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requires a change of about one milliampere in the current flowing through the coil (telephone relay); or the load may be an ohmic resistance of about 10 megohms, placed across the grid of a vacuum tube.

For a good photo-emissive vacuum cell (caesium oxide cell) the current, i, is a function of the applied voltage, E, divided by the sum, $R + r$, of the external and the internal resistance, the voltage drop produced across the load being equal to Ri. Provided that the cell always operates above saturation, the resistance, r, which it offers to small changes, the so-called a-c. or internal resistance, is much higher than that of any current-carrying device likely to be connected to the cell, and the current is independent of the applied voltage so that $i = gl$, where g, the current sensitivity, is constant and independent of l. The change in current, or in voltage, is proportional to the change in foot-candles or lumens, whatever the initial intensity of the light. In the best cells, giving about 50 μa. per lumen when exposed to the light from a tungsten filament at 2700° K., the current changes from about 3.5 μa at 10 ft.-candles to 3.2 or 3.9 μa. for a 10% increase or decrease in the illumination, and the power in a 10,000 ohm coil, used as a relay, varies by only a small fraction of a microwatt, an amount far too small to operate a sensitive relay. On the other hand, with an internal resistance of over 1000 megohms, the cell readily causes a drop of 3.5 volts across a 10 megohm resistor, a change which suffices for controlling the grid of a three-electrode tube over the linear range. The voltage change in the 10 megohm load amounts to 500 volts per lumen.

The current sensitivity, g, of a vacuum photocell is sometimes likened to the mutual conductance, G_m, or grid-plate conductance, of a radio tube, changes in illumination playing the part of changes in the grid voltage. On this basis the amplification factor, μ, of the radio tube corresponds to the change in plate voltage necessary for producing a small variation in the current, divided by the change in illumination which would cause the same variation in current in the absence of any external load. For those vacuum cells in which the sensitive surface is a solid piece of metal, in contrast to the modern cells with composite films—the silver-oxygen-caesium cell, for instance—the value of this factor lies beyond any definite limit, because the saturation current is clearly defined, and above saturation no change in anode voltage, however great, alters the current. When however the change in voltage across the load is considered, the output voltage sensitivity per lumen becomes equal to gR, an expression which corresponds to the voltage amplification, G, in a complete vacuum tube circuit. G, indeed, is equal to $\mu R/(R + r)$, or to $G_m R$ when the internal resistance r is very high (3).

In the *gas-filled photoelectric tube* the internal resistance decreases after the saturation stage has been passed, and ionization of the gas by the electrons, increasing according to an exponential law, has become appreciable. The current sensitivity may be raised to 5 or 10 times the value obtained in vacuum before the operation of the cell tends to become unstable and a given reading difficult to reproduce. In the tubes used in practice the change in current,

in microamperes, produced by a change in illumination of one lumen is of the same order as the change in milliamperes produced in a three-electrode tube when the grid bias varies by one volt. A change of one lumen corresponds to the difference between 1 ft.-candle and 1000 ft.-candles (the light intensity in the shade outdoors on a bright day), the entire range concerned in problems of illumination. The voltage sensitivity, μ, or amplification factor, of the cell assumes a finite value which varies, however, from one intensity, l, to another, since $g = \mu/r$ and r varies with the illumination. The output voltage sensitivity may be expressed as $\mu R/(R + r)$, where the magnitude of r now may drop to nearly that of R.

In the *barrier film cell* (cuprous-oxide-copper cell, selenium film cells, including the Photronic cell as a special case), the current obtained is strong, but an appreciable number of electrons, which the light has set free and forced through the barrier plane into the electrode, return immediately to their place of origin, forming a local short-circuit current, b, which is in parallel with the photoelectric current, i, flowing through the load, R. As long as the voltage drop in the load is quite small, or the load negligible, the relation between current, i, and illumination is given by the expression for a parallel circuit

$$i = i_0 \frac{r}{R + r} = \frac{kr}{R + r} l ,$$

where r is the internal resistance which the cell offers to the local short-circuit current. No external voltage is applied to barrier film cells; the metal which is in contact with the non-conducting film becomes the negative electrode (1, 6, 8). A comparison with the radio tube is no longer useful.

When the variation, dr/dl, in the resistance of the barrier plane, is negligible compared to r

$$\frac{di}{dl} = \frac{kr}{R + r} ,$$

and the output voltage sensitivity is R times this value, or when R is very large, equal to kr. The load, R, for which the power output reaches its highest value is found by studying the changes in i^2R with l; it is equal to r, whatever the value of l, and changes in power caused by changes in illumination are also largest at this value, namely equal to $k^2rl/2$.

For a rear-wall cuprous oxide cell, 1.5 in. in diameter, r may be made equal to 100 ohms, and k is equal to 100×10^{-6} amp. per lumen (7). The output voltage sensitivity thus is only 1/100 volt per lumen for $R = 10$ megohms. On the other hand, when the coil is used, a change of 10% in the illumination, assumed to be 10 ft.-candles, changes the photo-current by nearly 0.5 μa. The change obtained, without any external voltage applied to the cell, can be read on a portable instrument. The highest power sensitivity, $k^2rl/2$ watt per lumen when $r = R$, reaches $\frac{1}{2}$ microwatt per lumen. The maximum power output amounts to $k^2l^2r/4$ watts. Supersensitive relays of 300 ohms resistance require about 250 μa. for their operation; they are

capable of establishing contact for currents less than 0.2 amp. at six volts. One of the most sensitive relays of the galvanometer type works with 2μa. or 0.5 mv. and handles five watts at 40 volts with ohmic load.

In the general case, particularly in the selenium film cells, the total current, the sum of i and b, is not really proportional to the light received by the sensitive surface, owing to the voltage drop produced across the load, which tends to prevent all but the fastest electrons from crossing the barrier film. The load thus produces the same effect as an opposing potential applied to a cell carrying no load (5). In this case a certain voltage, V_0, is capable of suppressing the photoelectric current altogether. The voltage, V_0, depends on the highest velocities which the electrons possess after they are set free; it is of the same order, in practice, as the open-circuit voltage, namely a few tenths of a volt. The shorter the wave-length of the light that falls upon the cell, the higher is V_0. Moreover, the barrier film behaves as a rectifier rather than as a parallel resistance, r, to which the resulting potential drop is applied. For the Photronic cell the effect becomes appreciable when v exceeds 40 mv. (8). The resistance of the selenium film, finally, is so high that it must be taken into account by a resistance (ρ) placed in series with the external resistance, R (Fig. 1).

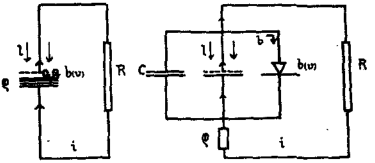

FIG. 1. *Actual circuit (left) and equivalent circuit (right) of a barrier-plane front-wall cell.*

If the decrease, caused by the counter e.m.f., in the number of electrons which succeed in traversing the barrier plane is assumed to conform to a straight line law from i_0, the value obtained at short circuit and equal to kl, down to zero when the circuit is open and the voltage v equal to V_∞, then

$$i = i_0\left(1 - \frac{v}{V_0}\right) = \frac{v}{R + \rho} + b(v)\,,$$

where $b(v)$ is governed by the rectifying properties of the barrier film. The voltage v corresponding to a given l may be determined from this equation, provided that the rectifying properties of the cell and the behavior of the short-circuited illuminated cell relative to a retarding potential applied to it have previously been established, and are available in the form of graphs or formulas. The current is R times smaller. In any case

$$v = \frac{i_0 - b(v)}{\frac{i_0}{V_0} - \frac{1}{R + \rho}}\,.$$

For an ideal rectifier $b(v)$ is a function Gv^x for positive values of v, and virtually zero, or equal to a small value, gv^y, for negative values of v. In the simplest case and for large changes in l, the exponent x is equal to unity, so that

$$v = \frac{kl}{\frac{kl}{V} + \frac{1}{R + \rho} + G}\,.$$

Hence the voltage produced is no longer proportional to the illuminatio
The ohmic load, R_h, giving the highest output of power,

$$R_h \doteq \rho + \frac{1}{\frac{kl}{V_0} + G} ,$$

is nevertheless independent of l, since k/V_0 is small. For very small valu of $(R + \rho)$, the current $i = v/(R + \rho)$ may be expressed as follows:

$$i = kl(1 - G(R + \rho)) - k^2l^2 \frac{R + \rho}{RV_0} Rl^2 = kl - KRl^2 .$$

Empirical formulas of this type agree with the measured values, l^2 bei replaced if necessary by l^n.

For the general type of barrier film cell, $b(v)$ is more accurately given Gv^2, where G is about 1/20 for the cuprous oxide, and 1/160 for the seleniu barrier cell. The voltage becomes

$$v = \frac{-\left(\frac{kl}{V_0} + \frac{1}{R + \rho}\right) + \sqrt{\left(\frac{kl}{V_0} + \frac{1}{R + \rho}\right)^2 + 4klG}}{2G} .$$

The resistance, ρ, which the electrons coming from the outside circuit mu overcome before they can complete their journey back to the barrier fil is of the order of 30 to 100 ohms in the case of selenium, and smaller f cuprous oxide cells (selenium having a specific resistance of $\frac{1}{4}$ to $\frac{1}{2}$ megoh cuprous oxide 600 to 100,000 ohm per cc. depending on traces of cupr oxide present.

When the last term under the root sign is much smaller than the squar and R larger than ρ, the current becomes equal to

$$i = kl - \frac{R + \rho}{RV_0} Rl^2 = kl - KRl^2 ,$$

as before. A similar formula in which l^2 is replaced by $l^{1.75}$ is valid between 2 and 250 ft.-candles for the Photronic type of selenium cell, as long as t load used at the stronger intensity does not exceed 700 ohms.

By computing $v^2R/(R + \rho)^2$ and differentiating with respect to R, t resistance, R, giving maximum power output for a given illumination, obtained by solving the equation.

$$\left(\frac{kl}{V_0} + \frac{1}{R + \rho}\right)^2 + 4klG - \frac{R^2}{(R^2 - \rho^2)^2} = 0 ,$$

or

$$R^4\left(4V_0G + \frac{kl}{V_0}\right) + 2R^3 - R^2\left(2\rho + \frac{3V_0}{kl} + 2\rho^2\left(4V_0G + \frac{kl}{V_0}\right)\right) - 2R\rho\left(\rho + \frac{V_0}{kl}\right) +$$
$$\rho^2\left(2\rho + \frac{V_0}{kl} + \rho^2\left(4V_0G + \frac{kl}{V_0}\right)\right) = 0$$

On introducing the values k and V_0 valid for selenium film cells ($k = 2 \times 10^{-}$ amp. per metre-candle or lux, $V_0 = 0.6$ volt) this expression reduces to

$$R_h = \sqrt{\frac{3}{4klG}}$$

for the entire range of illumination from the lowest values nearly up to the intensity outdoors in sunlight, about 100,000 metre-candles. When l is made 10 times larger, R must be made 3.17 times smaller, according to this formula, in order to secure the highest power. In practice an even greater reduction, between 5 and 7, is found necessary to obtain this result, indicating that the exponent x in the law Gv^x is probably larger than two. The empirical formula just mentioned gives

$$R_h = 0.24 V_0/sl^{3/4} ,$$

which corresponds to a ratio of 5.6 for the resistances R_h, when l is increased 10 times its value.

Though the selenium barrier cells have recently been improved so as to give more nearly reproducible readings while losing very little of their sensitivity, an important factor in meteorological work, the necessity of decreasing the resistance in order to obtain a high power output at high intensities is still a serious drawback when the direct conversion of light into electrical power is considered. The theory would indicate that in this respect the development work should aim at producing barrier films which rectify according to a law involving no higher powers of the voltage than the second.

At values of l below about 100 ft.-candles (1 ft.-candle = 10.76 metre-candles or lux), the values of R_h are quite large, and as the open circuit voltage, for $R = \infty$, is given by the expression $V_\infty = \sqrt{kl/G}$, and the short circuit current i_0 is equal to kl, the highest power output for a given illumination may be written as

$$L_h = 1.15\, i_0 V_\infty .$$

Taking the constants of a typical selenium-iron cell, 14 mm. in diameter, k between 10^{-8} and 2×10^{-8} amp. per metre-candle, $G = 1/160$, L_h has a value between 0.4 and 1.1 μw. at 1000 metre-candles against the measured value of 0.7 μw. (5). Reducing l to one-tenth or about 10 ft.-candles brings L_h down to 1/32 of its former value. The current obtained, however, is still appreciable, about 60 μa., so that a 10% change could be readily detected. The low output is due to the low voltage produced, only about 20 mv., whereas the normal vacuum photoelectric cell, in the absence of any external potential, would produce about 700 mv. between closely spaced electrodes, regardless of the illumination. But in this case the output is small, owing to the very high internal resistance.

Finally the change in the resistance, 1/G, of the photoconductive cells, which respond to the light almost like gas-filled photo-emissive cells, is proportional to the *nth* power of the illumination:

$$G = G + kl^n = 1/r = \frac{1 + kl^n r_0}{r_0} .$$

For selenium the value of n lies between one-quarter and one-half. The resistances in the dark range from one-half to several hundred megohms, depending upon the heat treatment which the selenium has undergone. The object of the treatment is to make G equal to at least $2G_0$ when l is about

10 ft.-candles. In the most sensitive cells G may be as high as 50 G_0 under the same conditions, so that the conductance in the dark may be neglected. From $i = E/(R + r)$ it follows that

$$\frac{di}{dl} = \frac{di}{dr}\frac{dr}{dl} = \frac{nkEl^{n-1}}{(R + r)^2\,(G_0 + kl^n)^2}\ ,$$

or when the load R is made equal to $r/2$ to ensure highest sensitivity

$$\frac{d(i^2R)}{dl} = \frac{d(i^2R)}{dr}\frac{dr}{dl} = \frac{2nkE^2Rr^2l^{n-1}}{(R + r)^3} = \frac{8nkE^2l^{n-1}}{27}\ .$$

Even when n is not less than 1/2, the sensitivity is very high in the lower range of illumination, and under these conditions, the photoconductive cell still remains virtually the only cell capable of directly operating ordinary telephone relays. The change in potential across high resistance cells also suffices for the control of electronic or ionic tubes.

In view of the many endeavors to improve the available photoelectric cells it is of interest to examine the maximum mechanical work which they may be expected to yield in the absence of an external electromotive force. During the brightest hours of the day, each square centimetre, when placed normally to the sun's rays, receives about 0.12 watt per sec. Of this energy, one-third lies in the visible, and another third between the visible and the wave-length 1.2 μ ; one-tenth is in the ultra-violet. The cell is not sensitive to radiation of wave-length greater than 1.2 μ. Even assuming complete absorption and transformation into mechanical energy, the incident energy could not actuate ordinary telephone relays without being amplified. Supposing that in the visible the average quantum amounts to 3.3×10^{-12} erg, whereas in reality it varies from 5×10^{-12} in the blue to 2.6×10^{-12} at 0.76 μ, and that one quantum sets one electron free, one watt of sunlight would produce a current of five amperes, so that neglecting space charges, the potential produced by the cell would be at the most 0.2 volt. With artificial sources of light, an incandescent body at 2710° K., for instance, the same degree of illumination as produced by the sun, 100,000 metre-candles, corresponds, of course, to a much larger amount of radiant energy, because the greater part of the visible radiation lies in a region to which the eye is several times less sensitive than to sunlight, and because the proportion of energy radiated between the visible and 1.2 μ is nearly one-third, in contrast to 1/12 in the visible. The output of photocells ought hence to be referred to the total incident energy.

Despite the capacities associated with the cells, the computed sensitivities, perhaps with the exception of those of the ordinary selenium resistance cell, are also valid for rapidly repeated changes in the intensity of illumination, provided that the frequency does not exceed 500 or 600 cycles per second. It is therefore possible to illustrate and study one of the main differences among the various cells, the great voltage sensitivity of the photo-emissive type and the great current sensitivity of the selenium barrier film cells, by using a telephone receiver as a load and exposing the cell to the light from

lamps operated on 60 cycles. Barrier film cells render the 120-cycle fluctuation in the light of the lamps, amounting to a few per cent, definitely audible: photo-emissive tubes require one stage of vacuum tube amplification; the same amplification would be useless when applied to barrier film cells.

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